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PLANETARY QUARANTINE

Semi-Annual Review
Space Research and Technology

1 Jan - 30 June 1971

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PREFACE

This document contains a report on Research and Advanced Development at the Jet Propulsion Laboratory during the period January 1, 1971 to June 31, 1971 sponsored by the Planetary Quarantine Branch of the NASA Office Space Science and Applications.

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SECTION I

SPACECRAFT CLEANING AND DECONTAMINATION TECHNIQUES

NASA No. 191-58-61-02-55

COGNIZANCE: H. W. Schneider, A. S. Irons

ASSOCIATE PERSONNEL: C. Hagen (AVCO), G. Simko (AVCO)

1.1 INTRODUCTION

The objective of this task is to develop methodology and procedures for the reduction of microbial burden on an assembled spacecraft at the time of encapsulation or terminal sterilization. This technology is required for reducing excessive microbial burden on spacecraft components for the purposes of either decreasing planetary contamination probabilities for an orbiter or minimizing the duration of a sterilization process for a lander.

1.2 SIGNIFICANT ACCOMPLISHMENTS

1.2.1 Mechanical Cleaning

During this reporting period, an experimental device (designated a vacuum flow simulator) was designed and fabricated to study the behavior of particles microscopically under different airflow conditions. The installation and calibration of the device was initiated. The overall test set-up is shown in Fig. 1-1.

The vacuum flow simulator is intended to simulate the cleaning aerodynamics of a spacecraft surface in a controllable and measureable manner while observing the test particulates through a microscope. A schematic of the apparatus is shown in Fig. 1-2. It consists of a plenum chamber (2) attached to the microscopic substage (1), a glass slide (3) which may be seeded with the test particles (4), and a test nozzle (or brush) (5), which can be moved up and down to vary the standoff from the sample surface. The entire device can be moved vertically by means of the existing focusing mechanism.

The atmospheric air (p_o , t_o) enters the system through a calibrated inlet and expands from the plenum chamber (2) through the narrow slot between the nozzle (5) and the seeded sample surface (4). The pressure head, to produce the flow-velocity and to cover losses, is provided by a vacuum pump connected downstream to the system. The flow rate through the system can be controlled by means of a throttling valve, and is determined by measuring the pressure drop (Δp_1) across the inlet nozzle by means of a slant tube water manometer. The surface pressure profile across the test nozzle (5) is measured by means of a precision ground and tapped steel plate connected to a mercury U-tube-manometer. (The steel plate replaces the glass slide during calibration tests.)

Figure 1-3 shows the test nozzle attachments used: (from left to right) a round convergent nozzle (5mm ID); a sharp edged nozzle (5mm ID); and a sable brush (6.5 mm OD). A circular shape was selected for all test attachments to avoid edge effects. Figure 1-4 shows a close up view of the convergent nozzle through the glass slide.

The objective of the test activity with the described apparatus is to determine the relationship between the parameters effecting the efficiency of vacuum cleaning, mainly the nozzle shape and standoff from the surface, the flow velocity, the conditions of the sample surface, and the size and adhesive properties of the particulates.

The parameter for evaluation will be the mean air velocity between the nozzle lip and the sample surface, which can be determined by means of the measured data using common thermo-aerodynamical relations.

The following techniques will be applied:

- (1) Acquiring a particle of interest, size its major dimensions and determine the mean detachment velocity or
- (2) Sweep the sample with a certain mean velocity and compare the counts taken prior to and after the sweep.

Tests to determine the basic aerodynamical characteristics of the apparatus are in progress.

1.2.2 Ethylene Oxide

The contractual effort was completed and a final report issued.

1.3 PROBLEM AREAS

No problem areas were noted during this reporting period.

1.4 FUTURE ACTIVITIES

During the next reporting period, tests using the vacuum flow simulator will be used to perform experiments to determine the removal efficiency of:

- 1) different test nozzles; 2) dry, wet, and fingerprinted dust samples; and
- 3) freon spray and dry nitrogen flow.

1.5 PUBLICATIONS

Becton, Dickinson Research Center, "Development of Parametric Data for the Establishment of an Ethylene Oxide Cycle for the Decontamination of Spacecraft," Final Report, July 1971.

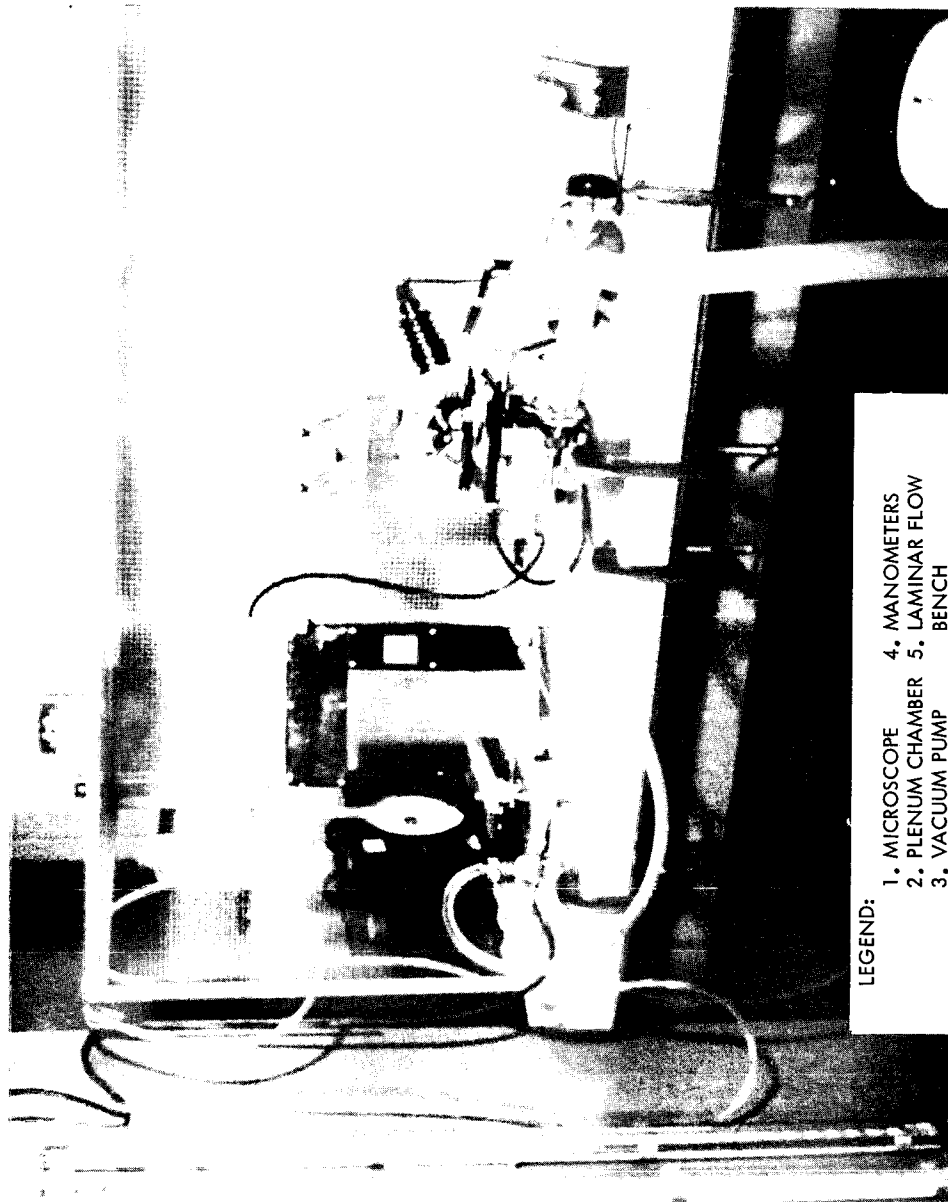


Fig. 1-1. Test set-up

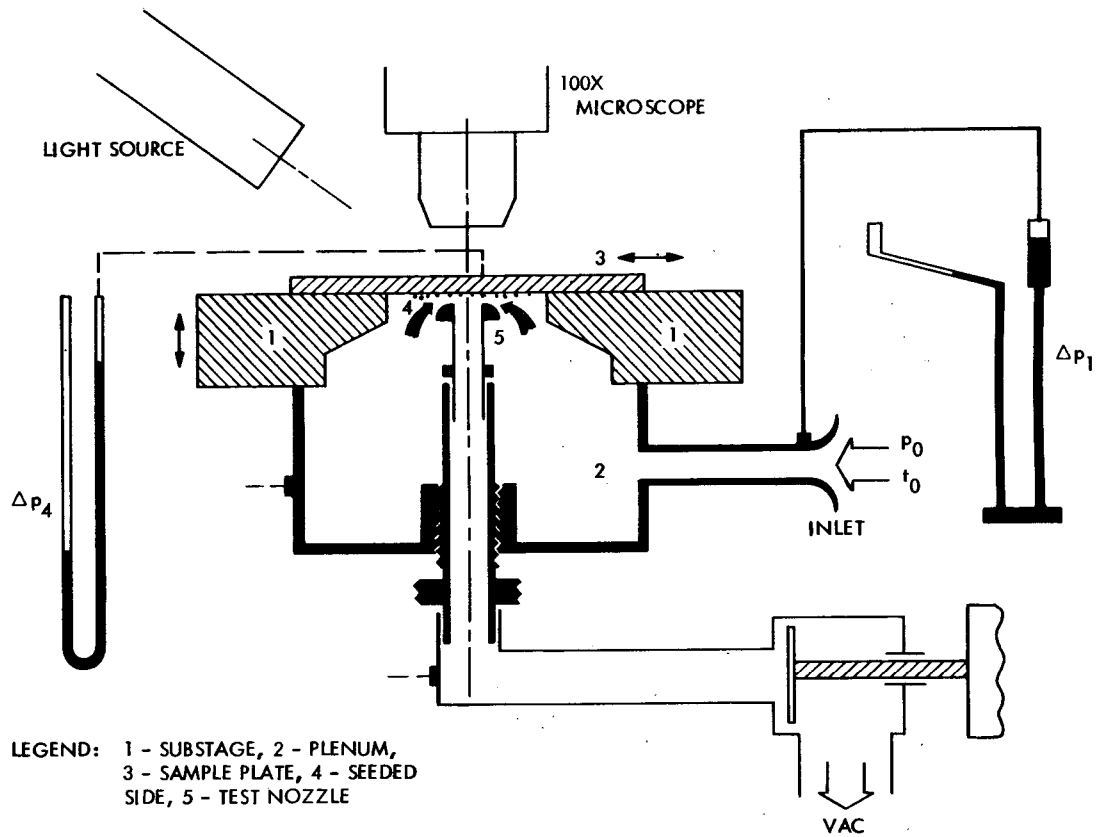


Fig. 1-2. Schematic of vacuum flow simulator

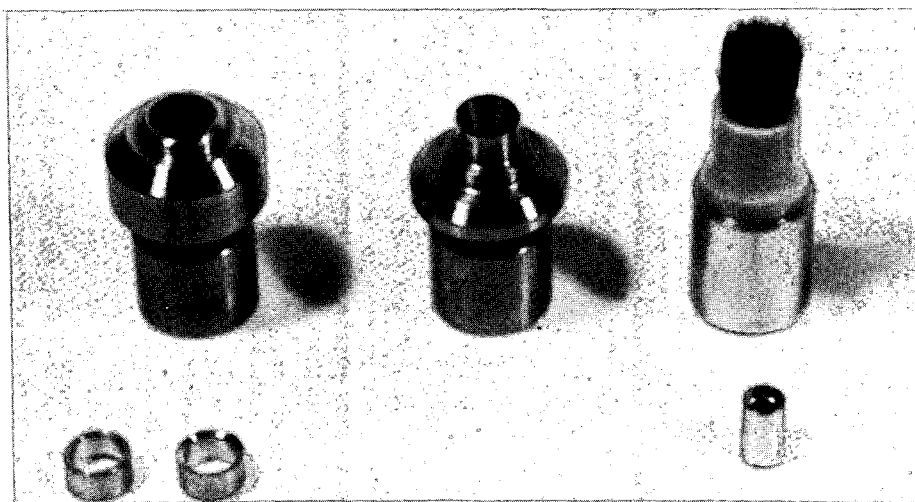


Fig. 1-3. Test nozzle attachments



Fig. 1-4. Convergent nozzle seen through glass slide

SECTION II

STUDIES OF SPACECRAFT STERILIZATION PARAMETERS

NASA No. 191-58-61-06-55

COGNIZANCE: M. D. Wardle

ASSOCIATE PERSONNEL: C. A. Hagen (AVCO), G. Simko (AVCO)

and R. C. Koukol (JPL)

2-1. INTRODUCTION

The primary objective of this task area is to acquire the necessary parametric information to define optimum flight acceptance and sterilization processes for space hardware. In line with this goal, the dry-heat resistances of microorganisms representative of those found on flight spacecraft are characterized in this section.

2.2 SIGNIFICANT ACCOMPLISHMENTS

2.2.1 Thermal Resistance of Microbial Populations Occurring in Hardware Assembly Areas

In conjunction with the microbiological monitoring program performed during the launch operations for Mariner-Mars 1971, an experiment was enacted to collect naturally occurring microbial fallout and estimate its dry-heat resistance. Large sheets of teflon 0.14 m^2 (1.5 ft^2) were deployed as collection surfaces in the Mariner spacecraft assembly areas at Cape Kennedy. After a 1-week exposure to the environment, the surfaces were collected and subjected to dry-heat cycles at $125^\circ\text{C} \pm < 1^\circ\text{C}$.

The extinction point for the microbial populations occurred between 9 and 13 hours of exposure to dry-heat (Table 2-1).

A method for converting extinction data to $D_{125^\circ\text{C}}$ values yielded the results shown in Table 2-2. This procedure for calculating D - values from fraction negative data produced worst case and average $D_{125^\circ\text{C}}$ values of 2.18 and 1.29 hours, respectively.

Table 2-1. Extinction point assay of AFETR fallout

Time, hr	No. of sites sampled	ft ²	Sites positive, %
1	12	18	83
3	24	36	50
6	23	34.5	22
9	19	28.5	5.3
13	42	63	0

The Mariner microbiological monitoring program included an environmental sampling phase which entailed the exposure of 1 x 2 in. stainless steel coupons to the spacecraft assembly environments. Table 2-3 shows a comparison of this environmental sampling to the results obtained using the large teflon surfaces. The order of magnitude of the populations detected compared quite favorably with the exception of the vegetative cells found in the north bay of the Explosive Safe Facility (ESF).

The prime objective of this experiment was to obtain an estimate of the dry-heat resistance of naturally occurring, non-cultured microbial populations. In order to more fully explain and relate the results acquired to the NASA sterilization program, it was decided to isolate, identify, and dry-heat test certain of those organisms surviving different time exposures at 125°C on the teflon sheets. The identification of these organisms was accomplished under a cooperative effort with the USPHS, Cape Kennedy. Dry-heat testing was conducted in ambient and GN₂ atmospheres. A modified oven capable of maintaining a O₂ level of less than 2.5%, when under constant GN₂ purge, was used for GN₂ testing. The same oven (minus GN₂ purge) was used for the ambient testing.

It was found that the dry-heat resistance of the isolates was approximately 2-4 times greater in air than in nitrogen (Table 2-4). As would be expected, the resistances of the cultured isolates were significantly less than their noncultured counterparts (Table 2-5).

Table 2-2. Calculation of $D_{125^{\circ}\text{C}}$ values from FN data

t Time, hr	r No. of replicates tested	p No. positive	q No. negative	N_t	N_o	$D_{125^{\circ}, \text{hr}}$
1	12	10	2	1.79	5.86×10^2	0.397
3	24	12	12	0.693	1.29×10^3	0.917
6	23	5	18	0.247	9.56×10^2	1.67
9	29	1	18	0.048	6.29×10^2	2.18
13	42	0	42	NA	8.33×10^2	NA
						$\bar{X} = 1.29$
N_t = Survivors per replicate unit = $\ln(r/q)$						
N_o = Estimate of initial spore burden						
$D_{125^{\circ}\text{C}} = \frac{t}{\log N_o - \log N_t}$						

Table 2-3. Numbers of viable organisms/ft² in AO and ESF facilities

Location	Spores		Vegetatives	
	Stainless steel	Teflon	Stainless steel	Teflon
ESF-North bay	1.85×10^2	2.04×10^2	1.45×10^4	1.58×10^3
ESF-Air lock	-	1.51×10^3	-	5.87×10^4
ESF-South bay	1.12×10^2	4.72×10^2	5.55×10^3	6.54×10^3
AO-High bay	4.60×10^1	3.97×10^1	1.02×10^3	8.69×10^2

Table 2-4. Dry-heat resistance of cape isolates

Isolate	Teflon exposure (hr)	D _{125° C} (min)	
		Air	Nitrogen
1	3	56	15
2	3	13	-
3	3	13	-
4	6	19	9
5	6	18	10
6	6	22	-
7	6	42	15
8	6	155	44
9	9	26	10

Table 2-5. Comparison of non-cultured vs cultured dry-heat resistance

Teflon exposure (hr)	D-value from FN data (min)	D-value in air for cultured isolates (min)
3	54	27
6	102	51
9	132	26

2.3 FUTURE ACTIVITIES

Further testing of teflon and spacecraft isolates will be reported in the next semi-annual review under the Spacecraft Decontamination and Cleaning tasks.

2.4 PUBLICATIONS

Wardle, M. D., Brewer, W. A., and Peterson, M. L., "Dry-Heat Resistance of Bacterial Spores Recovered from Mariner-Mars 1969 Spacecraft," Applied Microbiology 20:827-831, 1971.

2.5 PRESENTATIONS

Wardle, M. D., "Thermal Resistance of Microbial Populations Occurring in Hardware Assembly Areas," presented to AIBS Planetary Quarantine Advisory Panel, June 1971.

SECTION III

NATURAL SPACE ENVIRONMENTAL STUDIES

NASA No. 191-58-62-04-55

Cognizance: D. M. Taylor - M. Knittel

Associate Personnel: C. A. Hagen (AVCO), R. L. Olsen (Boeing)

C. D. Smith (AVCO), E. A. Gustan (Boeing)

3.1 INTRODUCTION

The objective of the Natural Space Environment Studies is to determine the probability of microorganisms surviving exposure to elements of space environment including launch pressure changes, vacuum and heat, and different types of solar, cosmic, and planetary trapped radiation. Twelve organisms, randomly selected from over two hundred isolates recovered at different assembly and test stages from Mariner Mars '71 spacecraft, and two comparative organisms, Bacillus subtilis var. niger and Staphylococcus epidermidis ATCC 17917, are being tested. Nine of the spacecraft isolates are spore-formers while three are nonsporeformers. These fourteen organisms are used in all experiments constituting a unified approach that examines the effects of a variety of factors of the natural space environment on the survival of a spacecraft microbial subpopulation.

Additional task areas are directed toward understanding the probability of release of microorganisms from the interiors of solids by simulated spacecraft impact and aeolian erosion and, if released, the probability of growing in the environment of a particular planet.

3.2 SIGNIFICANT ACCOMPLISHMENTS

3.2.1 Effect of Simulated Spacecraft Launch Pressure Changes on Survival of Microorganisms

3.2.1.1 Description of Study. The launch profile study is designed to examine the effect of rapid pressure changes, that may occur during a spacecraft launch,

on the survival of microorganisms. The fourteen test organisms previously described were exposed to changes in pressure similar to that recorded for the Mariner '69 spacecraft.

The organisms were exposed to the vacuum environment for 12 min. Additional exposures of one or 24 hr were conducted to determine what effect these extended vacuum exposures had on the survival of the organisms following the launch pressure change. Throughout this report the 12 min launch pressure change and the 12 min launch pressure change followed by either a 1 or 24 hr extended vacuum exposure are collectively referred to as vacuum exposures.

During the 12 min launch profile the organisms were exposed to 20°C while during the 1 and 24 hr vacuum exposures the organisms were exposed to both 20 and -20°C. With the 12 min launch profile the organisms experienced an approximate 10^{-7} torr pressure change when the pressure decreased from 750 torr to 10^{-5} torr. Order of magnitude changes to 10^{-6} and 10^{-7} torr occurred during the 1 and 24 hr vacuum exposures, respectively.

3.2.1.2 Materials and Methods. The organisms were recovered from the MM '71 PTM spacecraft at various microbiological assay milestones during the assembly and test of the spacecraft by use of standard procedures for the microbiological examination of spacecraft hardware. A swab sample was placed into 10 ml of sterile distilled water and was sonicated at 25 KHz for 2 min. The sample was then divided into two 5 ml portions with 4 ml of one portion being plated directly by the pour plate method using Trypticase Soy Agar (TSA; Baltimore Biological Laboratories). The three nonsporeformers and six sporeformers were derived from these samples. The remaining 5 ml portion was heat shocked at 80°C for 15 min and then 4 ml of this portion was plated by the pour plate method using TSA. Three sporeformers were derived from these samples.

The sporeforming isolates were sporulated in the liquid synthetic medium of Lazzarini and Santangelo (J. Bacteriol. 94:125-130, 1967) modified by the addition of 25 mg of both L-methionine and L-tryptophan to one liter of medium. The spores were washed 7 times with sterile distilled water and finally resuspended in 95% ethyl alcohol.

The nonsporeformers were maintained on TSA slants. A lawn was prepared by resuspending the growth from a slant in Trypticase Soy Broth (TSB; Baltimore Biological Laboratories) and inoculating TSA plates with this suspension. After a 48 hr incubation at 37°C, the cells were harvested and washed with distilled water for final resuspension in distilled water.

A planchet was inoculated with either 10^5 spores or 10^6 vegetative cells. The inoculum was allowed to air dry in a humidity-temperature controlled room (45% relative humidity, 21°C). The planchet was then exposed to the selected test conditions. At the conclusion of a test, the chamber was backfilled to ambient pressure with dry nitrogen. A planchet was placed into 10 ml of 0.1% peptone water and sonicated at 25 KHz for 12 min. The suspensions were serially 10-fold diluted with the appropriate dilutions plated in triplicate using TSA as the recovery medium. The plates were incubated at 37°C for 48 hr.

Figure 3-1 presents the pressure changes experienced by the microorganisms during a 12 min, 1 hr, and 24 hr vacuum exposure. For purposes of comparison the pressure change is included as experienced by the MM '69 spacecraft during launch. The pressure descent of the simulated launch profile during the initial 25 to 50 sec exceeded pressure changes experienced by the MM '69 spacecraft. The crossover point occurred at approximately 250-275 sec at 7×10^{-6} torr. By the end of 720 sec (12 min) the simulated launch profile pressure was approximately 3×10^{-6} torr. At 1 hr the pressure was 10^{-6} torr and at 24 hr it was 10^{-7} torr. The pressure profile of the simulated launch was reproducible throughout the experiments.

The data were subjected to an analysis of variance, and where statistical significance ($P < 0.01$) existed, the Duncan's Multiple Range test (Biometrics 11:1-42, 1955) was applied.

3.2.1.3 Results and Discussion. Table 3-1 shows the results from the analysis of variance performed on the nonsporeforming MM '71 isolates exposed to 12 min, 1 hr, and 24 hr vacuum exposures at 20°C. Isolate, time, and their interaction were significant. Table 3-2 shows the results obtained from applying Duncan's Multiple Range test to these data.

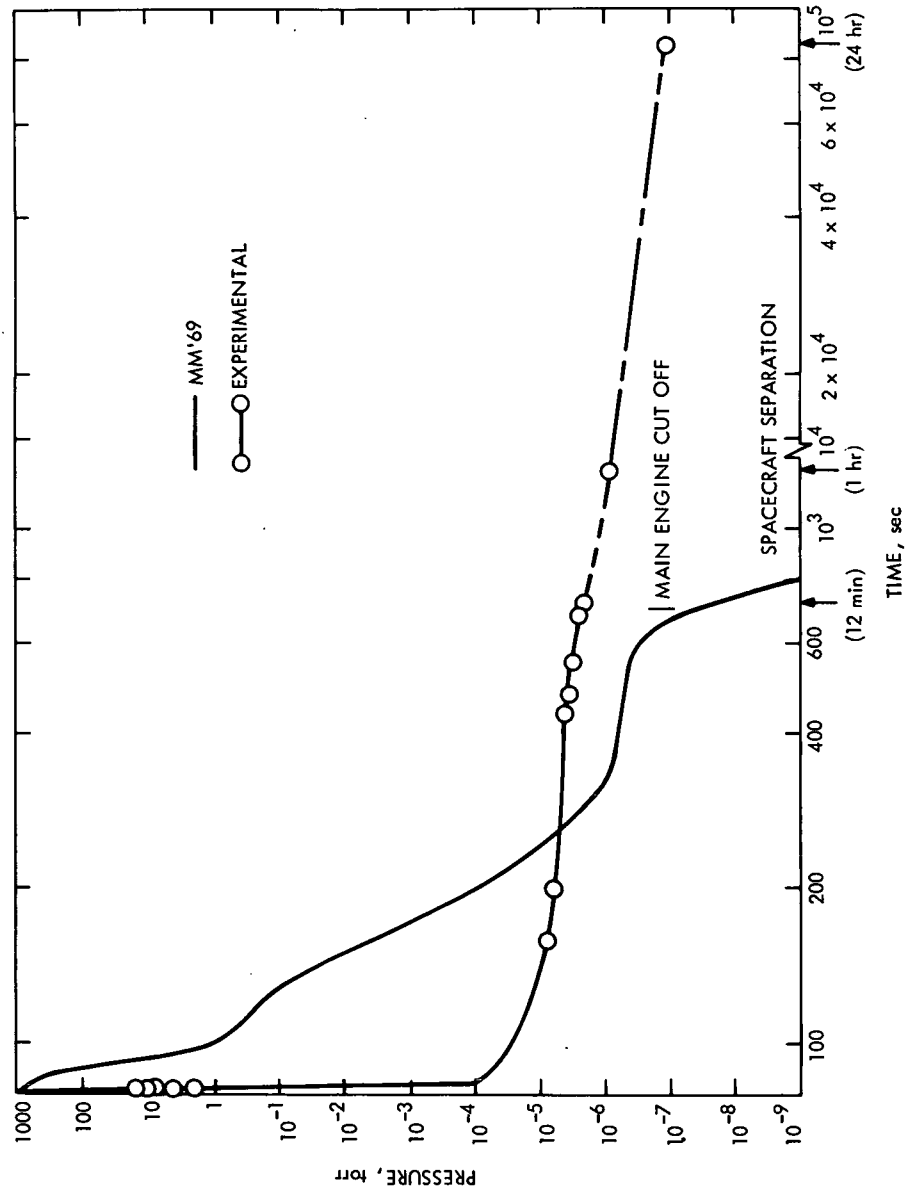


Fig. 3-1. Launch pressure profile

Table 3-1. Analysis of variance for nonsporeforming MM '71 isolates after vacuum exposures at 20°C

Source of variation	Degrees of freedom	Mean square	F ratio
Replicates	6	165	0.53
Treatment	11	2620	8.52**
A(Isolate)	3	4267	13.91**
B(Time)	2	1820	5.90**
AB	6	5610	18.27**
Error	66	308	
Total	83		

**F-ratios significant at $P < 0.01$ level.

Table 3-2. Comparison of percent survival of MM '71 nonsporeforming isolates after vacuum exposures at 20°C¹

Time	Isolate				Average time
	4	5	19	SE	
12 min	56 abcd	68 de	45 ab	75 def	61 _x
1 hr	70 d	106 g	40 a	92 fg	77 _y
24 hr	75 def	78 ef	65 bcde	48 abc	67 _x
Average isolate	67 _n	84 _o	50 _m	72 _n	

¹ Similar subscript letter indicates no significant difference between percent survivals.

The nonsporeforming MM '71 isolates exhibited significantly different resistances to the vacuum exposures. Isolate 19 was the least resistant to the pressure changes with 50% of the cells remaining viable while isolate 5 was most resistant with 84% of the cells remaining viable.

With respect to time, the 12 min vacuum exposure allowed fewest survivors (61%). The greatest percent survivors were recovered after the 1 hr vacuum exposure (77%) with the percent survivors recovered after the 24 hr vacuum exposure being intermediate (67%). At this time it appears that a rapid pressure descent followed, relatively close in time, by a pressure ascent which occurred with the 12 min vacuum exposure had the greatest effect on cell survival. The 67% survival found after 24 hr may reflect cellular death caused by extended vacuum exposure.

The analysis of variance performed on data obtained from the 1 and 24 hr vacuum exposures at both -20 and 20°C is shown in Table 3-3. Isolate, temperature, and the interactions isolate-time and isolate-time-temperature were significant at the $P < 0.01$ level. Table 3-4 shows the results obtained from applying Duncan's Multiple Range test to these data. Isolate 5 was the most resistant (99% survival) while isolate 19 was the least resistant (60% survival).

The significance of the interactions of isolate-time and isolate-time-temperature may be largely caused by a specific isolate's resistance to the experimental condition as with isolate 5. However, there appear to be sub-order interactions of significance with, for example, isolate SE which was less affected by the 1 hr than by the 24 hr vacuum exposure at both temperatures while with isolate 19 the 24 hr at -20°C test condition allowed greatest survival of the test conditions used.

Table 3-5 shows the results from the analysis of variance performed on the sporeforming MM '71 after 12 min, 1 hr, and 24 hr vacuum exposures at 20°C. Isolate, time, and their interaction were significant. Replication was also significant and indicated that some unknown event(s) occurred at the time the replicate experiments were performed.

There was an additional problem encountered with the sporeforming isolates. There were frequent occurrences when the number of spores

Table 3-3. Analysis of variance for nonsporeforming MM '71 isolates after 1 and 24 hr vacuum exposures at -20 and 20°C

Source of variation	Degrees of freedom	Mean square	F ratio
Replicates	6	825	1.59
Treatment	15	4278	8.23**
A(Isolate)	3	7760	14.92**
B(Time)	1	604	1.16
C(Temperature)	1	3866	7.43**
AB	3	11669	22.44**
AC	3	206	0.40
BC	1	456	0.88
ABC	3	17368	33.40**
Error	90	520	
Total	111		
**F-ratio significant at $P < 0.01$ level			

Table 3-4. Comparison of percent survival of MM '71 nonsporeforming isolates after 1 and 24 hr vacuum exposures at -20 and 20°C¹

Time Temp	Isolate				Time	Temp
	4	5	19	SE		
1 hr -20°C	78 bcd	110 d	44 ab	106 cd	80 _x	-20°C/84 _s +20°C/72 _t
+20°C	70 abc	106 cd	40 a	92 cd		
24 hr -20°C	106 cd	103 cd	85 cd	43 ab	76 _x	
+20°C	75 abcd	78 bcd	72 abc	48 ab		
Average Isolate	82 _n	99 _o	60 _m	72 _{mn}		
¹ Similar subscript letter indicates no significant difference between percent survivals.						

Table 3-5. Analysis of variance for sporeforming MM '71 isolates after vacuum exposures at 20°C

Source of variation	Degrees of freedom	Mean square	F ratio
Replicates	3	6528	6.79**
Treatment	29	4170	4.34**
A(Isolate)	9	2775	2.89**
B(Time)	2	5846	6.08**
AB	18	4682	4.87**
Error	87	961	
Total	119		
**F-ratios significant at P < 0.01 level			

recovered after exposure to the test environment exceeded the number of spores recovered before the exposure. Similar results have been reported for other deep space vacuum simulation experiments (A. A. Imshenetsky and S. V. Lysenko, Life Sciences and Space Research, Vol. III, pp 142-148, 1965; G. J. Silverman, Planetary Quarantine, edited by Hall, 1971; and E. A. Gustan and D. A. Strandberg, Boeing Company Report D2-121029-1, 1970). In our test the condition was particularly manifested with isolates 2, 12, 13, 16, and 18. Two possible explanations may be given for this problem: 1) the spore suspensions were comprised largely of clumps of 2 or more spores and that exposure to the test environments physically broke up the clumps; or 2) the spores were physically activated to germinate as a consequence of their exposure to the test environments.

Table 3-6 shows the results obtained from applying Duncan's Multiple Range test to the percent survival of MM'71 sporeforming isolates after vacuum exposures at 20°C. The sporeforming isolates exhibited significantly different resistances to the vacuum exposures. Isolates 1 and 8 were the least resistant with 94 and 91% survivals, respectively, while isolates 12, 13, and 18 were the most resistant and spores of the comparative organism, B. subtilis var. niger, were of intermediate resistance.

With respect to time the sporeforming isolates exhibited a different survival pattern than the nonsporeforming isolates. The 1 hr vacuum exposure allowed fewest survivors (98.5%) of the sporeforming isolates while no statistical significance existed between the percent survivors from either the 12 min (115.5% survivors) or 24 hr (122% survivors) vacuum exposures.

It should be emphasized that the resistance of isolates 12, 13, and 18 to the test conditions may be largely the result of the breaking up of spore clumps or spore germination by pressure changes rather than a true resistance. These same statements need also be considered in the interpretation of data from Table 3-8.

Table 3-7 shows the results from an analysis of variance performed on percent survival data of sporeforming isolates after 1 and 24 hr vacuum exposures at both -20 and 20°C. Isolate, time, and the interaction isolate-time, isolate-temperature, and isolate-time-temperature were significant.

The results from applying Duncan's Multiple Range test to these data are shown in Table 3-8. Isolates 1, 8, and 11 were least resistant to the test

Table 3-6. Comparison of percent survival of MM '71 sporeforming isolates after vacuum exposures at 20°C¹

Time	Isolate										Average time
	1	2	8	9	11	12	13	16	18	BSN	
12 min	97 abcd	97 abcd	93 abcd	106 abcd	136 de	170 ef	93 abcd	121 cde	140 de	102 abcd	115.5 _y
1 hr	70 abc	109 abcd	122 cde	108 abcd	75 abc	67 ab	117 bcd	101 abcd	75 abc	141 de	98.5 _x
24 hr	116 bcd	129 de	59 a	90 abcd	101 abcd	132 de	168 ef	117 bcd	208 f	100 abcd	122.0 _y
Average isolate	94 _{mn}	111 _{nopq}	91 _m	101 _{mno}	104 _{mnop}	123 _{pqr}	126 _{qr}	113 _{nopq}	141 _r	114 _{opq}	

¹ Similar subscript letter indicates no significant difference between percent survivals.

Table 3-7. Analysis of variance for sporeforming MM '71 isolates after
1 and 24 hr vacuum exposures at -20 and 20°C

Source of variation	Degrees of freedom	Mean square	F ratio
Replicates	3	1991	0.16
Treatment	39	5387	2.90**
A(Isolate)	9	9420	5.06**
B(Time)	1	24927	13.39**
C(Temperature)	1	351	0.19
AB	9	8797	4.73**
AC	9	13091	7.03**
BC	1	299	0.16
ABC	9	14058	7.55**
Error	117	1861	
Total	159		
**F-ratios significant at $P < 0.01$ level			

Table 3-8. Comparison of percent survival of MM '71 sporeforming isolates after 1 and 24 hr vacuum exposures at -20 and 20°C¹

Time Temp	Isolate										Average time
	1	2	8	9	11	12	13	16	18	BSN	
1 hr -20°C	69 abc	95 a→d	108 a→f	80 abc	74 abc	75 abc	120 a→f	103 a→e	131 a→f	128 a→f	98 _x
	70 abc	108 a→f	122 a→f	107 a→e	75 abc	67 abc	116 a→f	101 a→e	75 abc	140 cdef	
24 hr -20°C	119 a→f	134 b→f	56 a	128 a→f	77 abc	170 efg	182 fg	105 a→e	174 efg	115 a→f	123 _y
	116 a→f	128 a→f	59 ab	89 abc	74 abc	131 a→f	167 defg	117 a→f	222 g	100 a→e	
Average isolate	94 _{mno}	117 _{no}	87 _{mn}	102 _{mno}	75 _m	111 _{no}	147 _{pq}	107 _{no}	151 _q	121 _{op}	
¹ Similar subscript letter indicates no significant difference between percent survivals											

conditions with 94, 87, and 75% survivors, respectively, while isolates 2, 12, 13, and 18 were most resistant with 117, 111, 147, and 151% survivors, respectively. A greater number of spores survived the 24 hr vacuum exposure (123%) than the 1 hr vacuum exposure (98%).

It is recommended that experiments be performed to resolve the problem area encountered with the sporeforming isolates. These experiments could consider physical methods of germinating spores such as heat shock or chemical methods such as a defined synthetic germination medium containing adenosine-glucose-L-alanine to determine whether the pressure changes associated with the launch profiles affected spore germination.

3.2.1.4 Conclusion. Certain conclusions can be drawn from these data even though a problem exists with assaying sporeforming isolates. These conclusions are: 1) although significant in particular instances, initial populations of the single isolate most sensitive to the pressure changes were reduced only 0.5 log while the more resistant isolates were unaffected by the pressure changes; and 2) pressure change, like most physical or chemical agents, elicited a spectrum of resistances and emphasizes the requirement of testing as many isolates as possible before stating conclusions.

3.2.2 Effect of Ultra-High Vacuum and Heat on Survival of Microorganisms

The following is a modification of the abstract of an article accepted for publication in Space Life Sciences:

A Space Molecular Sink Research Facility (Molsink) was used to evaluate the ability of microorganisms to survive the vacuum of outer space. This facility could be programmed to simulate flight spacecraft vacuum environments at pressures in the 10^{-10} torr range and temperatures (-124 to 60°C) closely associated with surface temperatures of inflight spacecraft.

Initial populations of Staphylococcus epidermidis and a Micrococcus sp. were reduced approximately 1 log while exposed to -105 and 34°C, and approximately 2 logs while exposed to 59°C for 14 days in the vacuum environment.

Initial populations of the Micrococcus sp. were reduced 0.5, 0.8 and 1.6 logs during a 28-day exposure to -105, 34, and 59°C, respectively.

Spores of Bacillus subtilis var. niger were less affected by the environment. Initial spore populations were reduced 0.2, 0.3, and 0.8 log during the 14-day vacuum exposure at -124, 34, and 59°C, respectively.

3.2.3 Effect of Space Radiation on Survival of Microorganisms

3.2.3.1 Description of Study. The radiation study seeks to determine the effects of radiation, present in the natural space environment and planetary trapped radiation belts, on microorganisms associated with unsterilized spacecraft. The present study is limited to an evaluation of the Jovian trapped radiation belt, Table 3-9, and the low energy (3 keV) solar wind protons. The selection of dose rate and dose was based on minimum, nominal, and maximum models derived from previous analytical studies (The Planet Jupiter, NASA Monograph SP-8069, 1970).

The test organisms, isolated from MM '71 spacecraft, are the same organisms that were used in the launch pressure profile task. The radiation experiments are being conducted in a 10^{-6} torr vacuum at 20 and -20°C to determine any temperature effect on the survival of irradiated cells. During the reporting period of this study only the results of the effect of 2 MeV electrons will be reported.

3.2.3.2 Experimental Conditions. The experimental conditions described in the following sections will be the same for all radiation studies except for the radiation section which will change to reflect the type particle and energy being studied.

1. Microbiology. The derivation, culture, test, and assay procedures for the bacterial isolates are described in para. 3.2.1.2.

A photograph of the plachets and test fixture appears in the Semi-Annual Review, September, 1971, as Fig. 3-4, on page 3-18.

The radiation tests required certain procedures not required for the launch profile study. Because the radiation facilities were located at a distance from where the microbiology was performed, it was necessary for the test fixtures, with inoculated plachets, to be transported between radiation and

Table 3-9. Test matrix for electron and proton radiation of microorganisms

Energy (MeV)	Dose rate (particles $\text{cm}^{-2} \text{sec}^{-1}$)	Dose (Krad)
2	10^9	300
	10^{10}	150, 300, 450
	10^{11}	300
12	10^9	300
	10^{10}	150, 300, 450
	10^{11}	300
25	10^9	300
	10^{10}	150, 300, 450
	10^{11}	300

microbiology facilities before and after exposure. This was accomplished by placing the test fixtures with inoculated planchets into a sealable box fitted with inlet and outlet valves. The planchets were covered with aluminum foil and the box sealed. The box was purged with dry nitrogen for two minutes with both inlet and outlet valves open. Both valves were closed after the purging operation.

The vacuum chamber was also purged with dry nitrogen during the placement and removal of the test fixtures which occurred before and after radiation exposure.

Another procedure instituted to minimize possible oxygen and vacuum effects on radiation survival was to evacuate the chamber to 10^{-6} torr and maintain this vacuum for 0.5 hr prior to radiation exposure.

A block type of experimental design was used in the performance of the tests. Each test fixture held planchets individually inoculated with each of the fourteen test organisms. One test fixture was maintained at 20°C and another fixture at -20°C throughout the test. Control test fixtures were treated in a similar manner but were not irradiated. Each test condition, type radiation, radiation dose, dose rate, and energy, was performed four times. The planchets were assayed for viable bacteria in the same manner as the launch profile planchets, para. 3.2.1.2.

Data were analyzed with analysis of variance test and, where statistical significance existed ($P < 0.05$), Duncan's Multiple Range test was applied. A logarithmic transformation of the test data was performed before being subjected to an analysis of variance for all analyses involving the effect of different doses.

2. Vacuum Equipment. A photograph of the vacuum equipment used in this study appeared as Fig. 3-3 in the Semi-Annual Review, September, 1971. A description of the chamber, its operation, and capabilities, also was presented at that time. For the 2 MeV electron study a 5 inch diameter, 0.004 inch thick titanium foil window was mounted in the test vacuum chamber to allow passage of the electrons.

3. Radiation. The 2 MeV electrons were produced by the JPL dynamitron. Prior to the performance of the actual radiation experiments, a two-dimensional flux map was obtained at the test plane. The map was accomplished, with the 0.004 inch titanium window in place, through the use of two matched Faraday cups (0.67 cm^2 aperture), one fixed on the beam axis and the other mounted on a mechanical goniometer. It was determined that the flux decreased to 10% of maximum at the annulus or sample location. These results were obtained for an accelerating voltage of 2.1 megavolt, which provided, after a calculated energy loss in the window, 2.0 MeV electrons at the test fixture containing the microorganisms. Calculations of the dose rate in the

test annulus in terms of the flux in the beam center were performed in the form

$$\dot{D}(\text{rad/s}) = 1.6 \times 10^{-8} \frac{dE}{dX} (\text{MeV} - \text{cm}^2/\text{g}) \frac{\phi_0 (\text{e/cm}^2 \text{s})}{10}$$

where dE/dX is the stopping power of tissue for 2 MeV electrons and ϕ_0 is the flux in the beam center. ϕ_0 was continuously monitored during the exposures by a Faraday cup mounted in the center of the flange that held the test fixture. The Faraday cup output was also electrically integrated to provide the dose for each aperture.

The test matrix for 2 MeV electrons is shown in Table 3-10.

3.2.3.3 Results. The analyses of variance performed on the test data from 2 MeV electron radiation of spacecraft sporeforming isolates are shown in Tables 3-11 and 3-12. The effects of 150, 300, and 450 Krad exposures at $10^{10} \text{ e cm}^{-2} \text{ sec}^{-1}$ dose rate on the isolates are shown in Table 3-11. Isolate, temperature, dose, and the interaction isolate-dose were significant at the $P < 0.01$ level. An approximate one log reduction in survival occurred with the sporeforming isolates for each 150 Krad exposure, i.e., an increase of dose from 150 Krad to 300 and 450 Krad resulted in population decreases of 1 and 2 log(s), respectively. The effect of dose and temperature for BSN is shown in Fig. 3-2.

Of additional interest was the difference in radiation resistances of the isolates with respect to the comparative organism, B. subtilis var. niger. The radiation resistances of the isolates were greater at 20°C than at -20°C. At 20°C seven of the isolates were more resistant than B. subtilis var. niger to 150, 300, and 450 Krad, Fig. 3-3, while at -20°C two, three, and eight were more resistant than B. subtilis var. niger to the respective doses, Fig. 3-4, indicating a strong interaction of the relative resistance of the isolates depending on temperature and dose.

Table 3-10. Test matrix for 2 MeV electron radiation tests

Number of runs	Dose rate, e cm ⁻² sec ⁻¹	Fluence e cm ⁻²	Approximate exposure time, min	Dose, ¹ Krad
4	10 ¹⁰	9.7 x 10 ¹²	16	300
4	10 ¹⁰	4.85 x 10 ¹²	8	150
4	10 ¹⁰	1.45 x 10 ¹³	24	450
4	10 ¹¹	9.7 x 10 ¹²	2	300
4	10 ⁹	9.7 x 10 ¹²	160	300
¹ All dosages are based on absorption in carbon				

The analysis of variance on the effect of dose rate of 2 MeV electrons is given in Table 3-12. In this case the dose was held constant at 300 Krad and the dose rate was varied by exposing the organisms at 10⁹, 10¹⁰, 10¹¹ e cm⁻² sec⁻¹. The analysis shows that there was a significant difference (P < 0.01 level) due to dose rate. The effect of the three dose rates on percent survival across all sporeforming isolates is shown in Fig. 3-5. It was found that a dose rate of 10¹⁰ e cm⁻² sec⁻¹ resulted in a significantly lower percent survival than either 10⁹ or 10¹¹ e cm⁻² sec⁻¹. At 20°C eight of the isolates were more resistant than B. subtilis var. niger to electron fluxes of 10⁹, 10¹⁰, and 10¹¹ e cm⁻² sec⁻¹, Fig. 3-6, while at -20°C nine, three, and nine isolates were more resistant to the same respective fluxes, Fig. 3-7.

The analyses of variance performed on the test data from 2 MeV electron radiation of spacecraft nonsporeforming isolates are shown in Tables 3-13 and 3-14. The effects of 150, 300, and 450 Krad exposures at 10¹⁰ e cm⁻² sec⁻¹ dose rate on the isolate are shown in Table 3-13. Isolate and dose were significant at the P < 0.01 level. The effect of dose and temperature on S. epidermidis is presented in Fig. 3-8. Temperature was not significantly different at the P < 0.05 level.

Table 3-11. Analysis of variance for sporeforming MM'71 isolates exposed to 140, 300, and 450 Krad of 2 MeV electron radiation at $10^{10} \text{ e}^- \text{ cm}^{-2} \text{ sec}^{-1}$ dose rate¹

Source of variation	Degrees of freedom	Mean square	F ratio ²
Replicates	3	0.71	7.39**
Treatment	59	3.73	38.85**
A(Isolate)	9	1.52	15.87**
B(Temperature)	1	5.61	58.48**
C(Dose)	2	96.87	1010.15**
AB	9	0.14	1.44
AC	18	0.22	2.26**
BC	2	0.04	0.39
ABC	18	0.09	0.95
Error	177	0.10	
Total	239		

¹ A logarithmic transformation of the test data was performed before applying the analysis of variance.

^{2**} Significant at the $P < 0.01$ level.

Table 3-12. Analysis of variance for sporeforming MM'71 isolates exposed to 300 Krad of 2 MeV electron radiation at 10^9 , 10^{10} , and 10^{11} e cm⁻² sec⁻¹ dose rate

Source of variation	Degrees of freedom	Mean square	F ratio ¹
Replicates	3	1.28	0.70
Treatment	59	4.99	2.75**
A(Isolate)	9	20.03	11.05**
B(Temperature)	1	15.43	8.52**
C(Dose rate)	2	12.45	6.87**
AB	9	1.68	0.93
AD	18	1.50	0.83
BD	2	0.22	0.01
ABD	18	1.74	0.96
Error	177	1.81	
Total	239		
¹ ** Significant at the P<0.01 level			

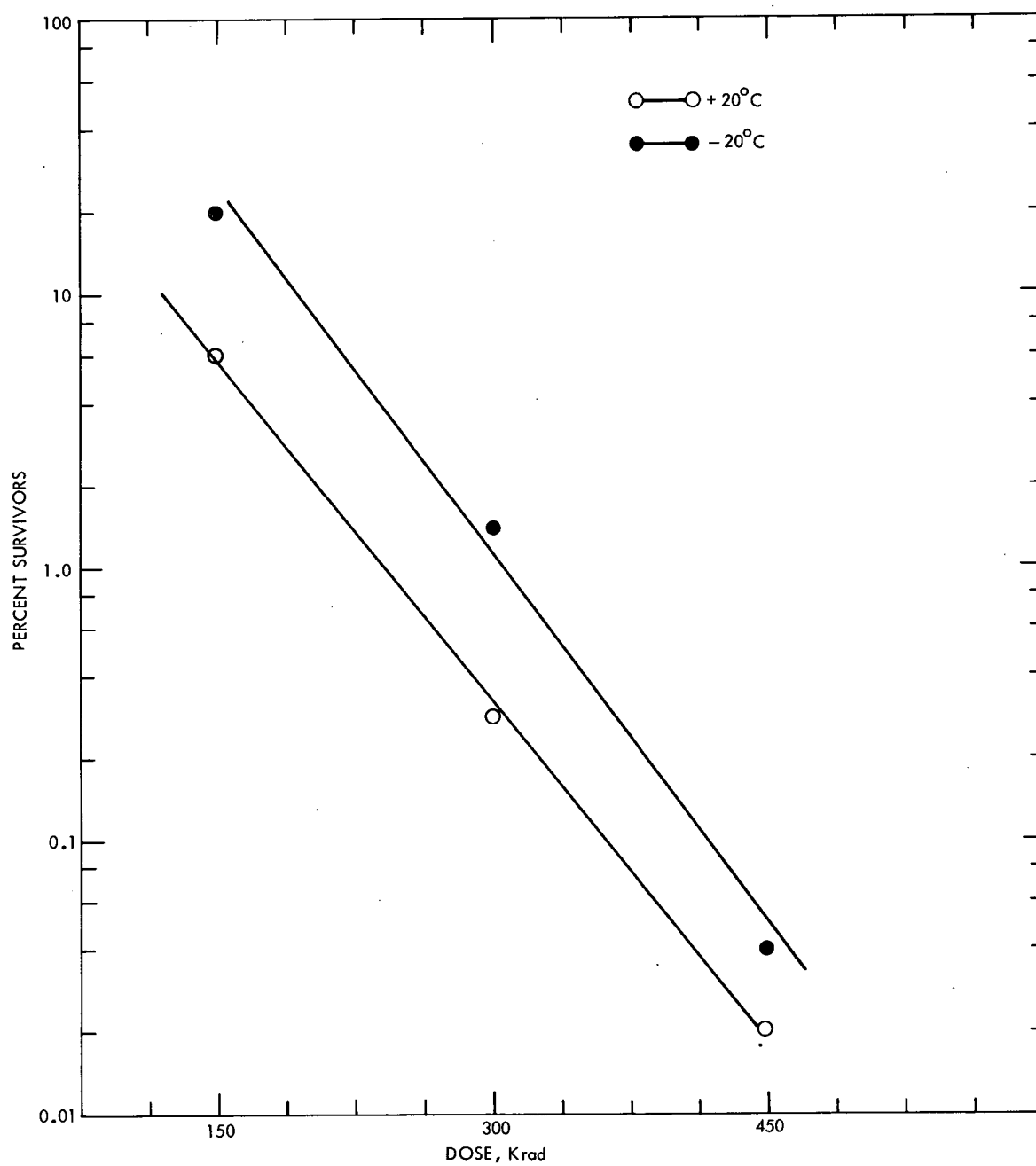


Fig. 3-2. Effect of temperature and dose of 2 MeV electrons at $10^{10} \text{ e cm}^{-2} \text{ sec}^{-1}$ dose rate on Bacillus subtilis var. niger spores

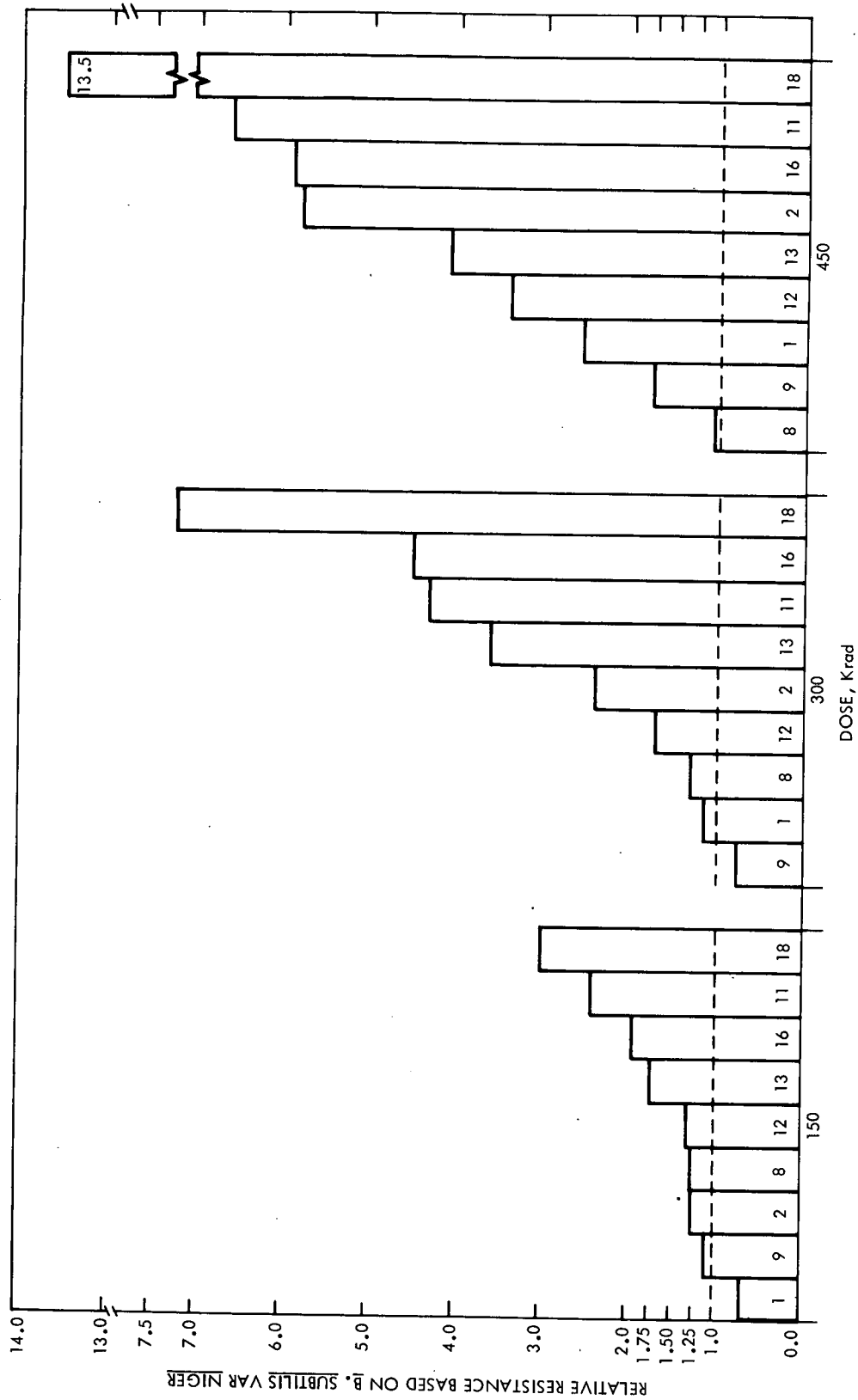


Fig. 3-3. Relative resistance of sporeforming isolates to 2 MeV electrons at different doses at 20°C

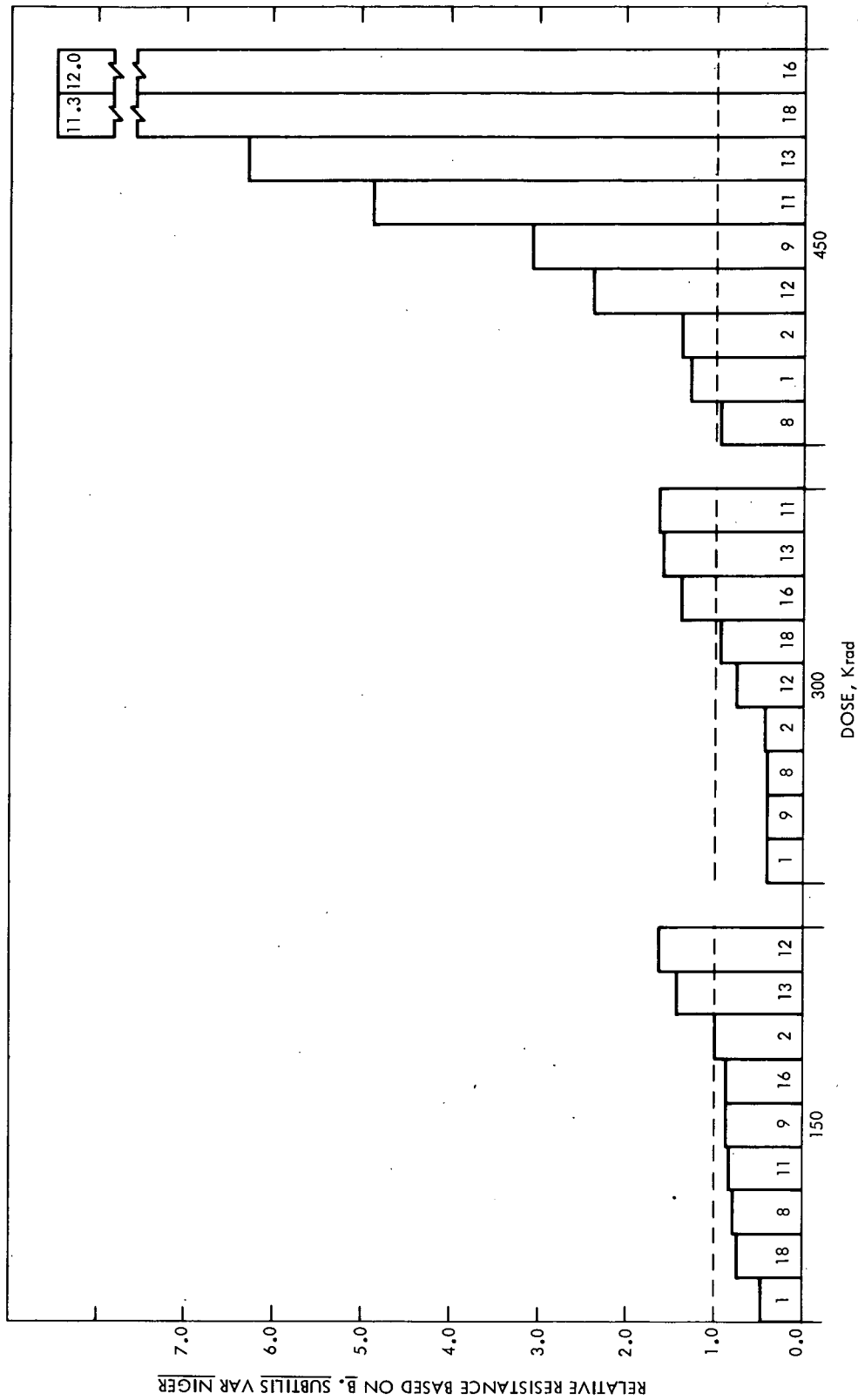


Fig. 3-4. Relative resistance of sporeforming isolates to 2 MeV electrons at different doses at -20°C

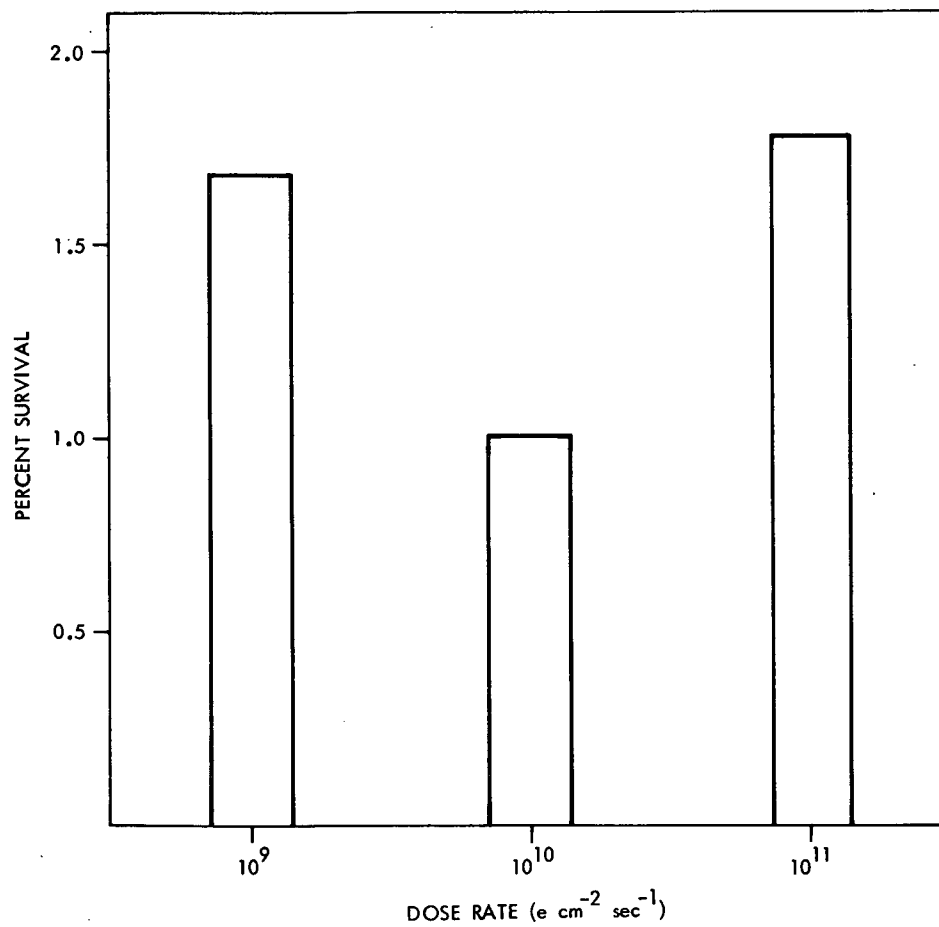


Fig. 3-5. Effect of 300 Krad exposure at different dose rates on sporeforming isolates

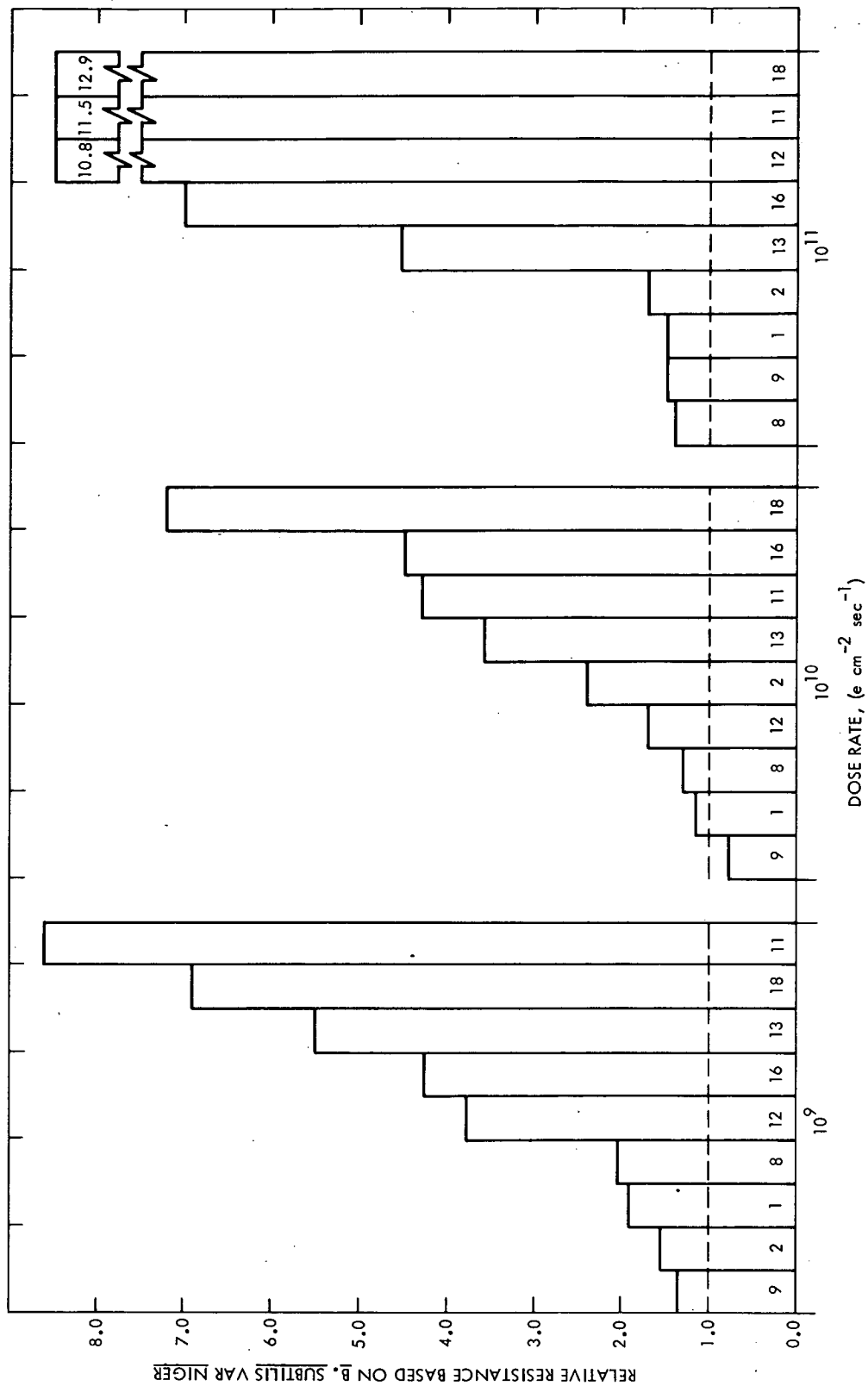


Fig. 3-6. Relative resistance of sporeforming isolates to 2 MeV electrons exposed to 300 Krad at different dose rates at 20° C

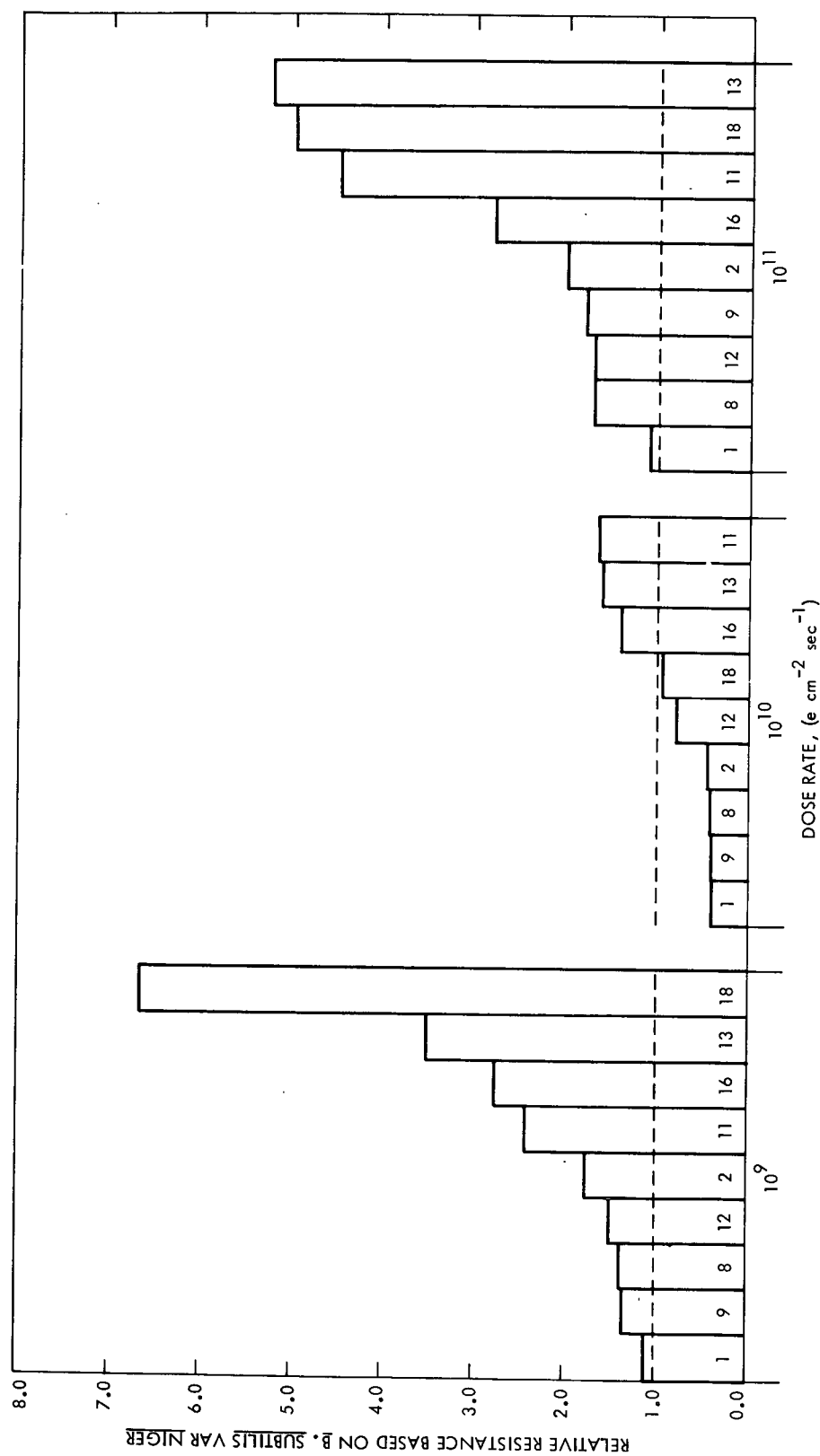


Fig. 3-7. Relative resistance of sporeforming isolates to 2 MeV electrons exposed to 300 Krad at different dose rates at -20°C

Table 3-13. Analysis of variance for nonsporeforming MM '71 isolates exposed to 150, 300, and 450 Krad of 2 MeV electron radiation at 10^{10} e cm^{-2} sec^{-1} dose rate¹

Source of variation	Degrees of freedom	Mean square	F ratio ²
Replicates	3	1.15	0.91
Treatment	23	13.86	
A(Isolate)	3	39.15	31.05**
B(Temperature)	1	1.06	0.84
C(Dose)	2	87.59	69.46**
AB	3	2.64	2.09
AC	6	1.15	0.91
BC	2	2.61	2.07
ABC	6	0.83	0.66
Error	69	1.26	
Total	95		

¹ A logarithmic transformation of the test data was performed before subjecting to the analysis of variance.

^{2**} Significant at the $P < 0.01$ level

Table 3-14. Analysis of variance for nonsporeforming MM'71 isolates exposed to 300 Krad of 2 MeV electron radiation at 10^9 , 10^{10} , and 10^{11} e cm⁻² sec⁻¹ dose rates

Source of variation	Degrees of freedom	Mean square	F ratio ¹
Replicates	3	5.68	2.52
Treatment	23	21.81	9.66**
A(Isolate)	3	107.79	47.76**
B(Temperature)	1	18.05	8.00**
D(Dose rate)	2	8.01	3.55
AB	3	15.41	6.83**
AD	6	9.35	4.14**
BD	2	4.59	2.03
ABD	6	5.13	2.27
Error	69	2.26	
Total	95		
¹ ** Significant at the P<0.01 level			

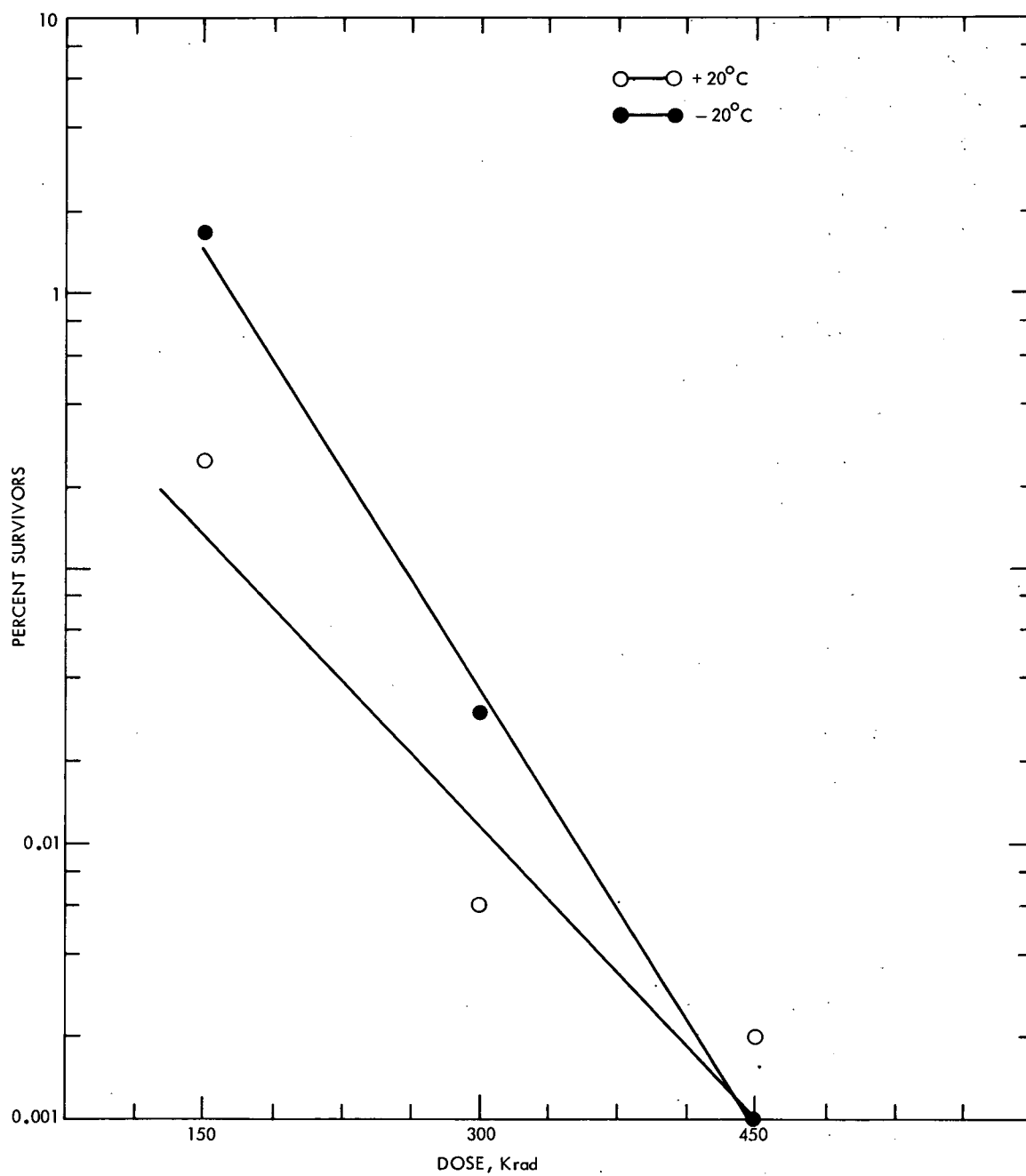


Fig. 3-8. Effect of temperature and dose of 2 MeV electrons at Staphylococcus epidermidis

The relative resistances of the nonsporeforming isolates were compared to S. epidermidis, Fig. 3-9. It should be noted that the relative resistances are on a logarithmic scale because of the extremely high relative resistances. All of the nonsporeforming isolates were more resistant than the comparative organism, S. epidermidis.

Table 3-14 presents the results from the analysis of variance performed on test data from dose rates of 10^9 , 10^{10} , and 10^{11} e cm⁻² sec⁻¹ at 300 Krad. Isolate, temperature, and the interactions isolate-temperature and isolate-dose rate were significant at the $P < 0.01$ level. Dose rate was significant at the $P < 0.05$ level. The effect of dose rate on nonsporeforming isolates was similar to that found with the sporeforming isolates. A dose rate of 10^{10} e cm⁻² sec⁻¹ had the lowest percent survival.

The relative resistances of the nonsporeforming isolates with respect to dose rate at 300 Krad were compared to S. epidermidis, Fig. 3-10. The relative resistances of the isolates exposed to 10^{11} e cm⁻² sec⁻¹ at 20°C were not included because no viable cells of S. epidermidis were recovered after exposure to the test conditions. All of the nonsporeforming isolates were more resistant to the dose rates than S. epidermidis. Isolate 5, a yellow pigmented micrococcus, was considerably resistant to the test conditions.

The radiation resistance to 2 MeV electrons of isolate 5 was similar to the sporeforming isolates. Table 3-15 shows the relative resistance of this isolate when compared to irradiated spores of B. subtilis var. niger. The relative resistance was found to vary from 0.54 to 21.5 depending on the test conditions.

3.2.4 Probability of Growth

The probability of growth is directed towards evaluating and understanding the maximum and minimum limits of environmental parameters such as temperature, pressure, availability of moisture, and atmospheric composition for microbial growth and to relate this information to possible microbial growth in extraterrestrial environments. A literature review was conducted during the current reporting period to establish maximum and minimum limits of selected environmental parameters permitting growth of bacteria. This material is presented at this time.

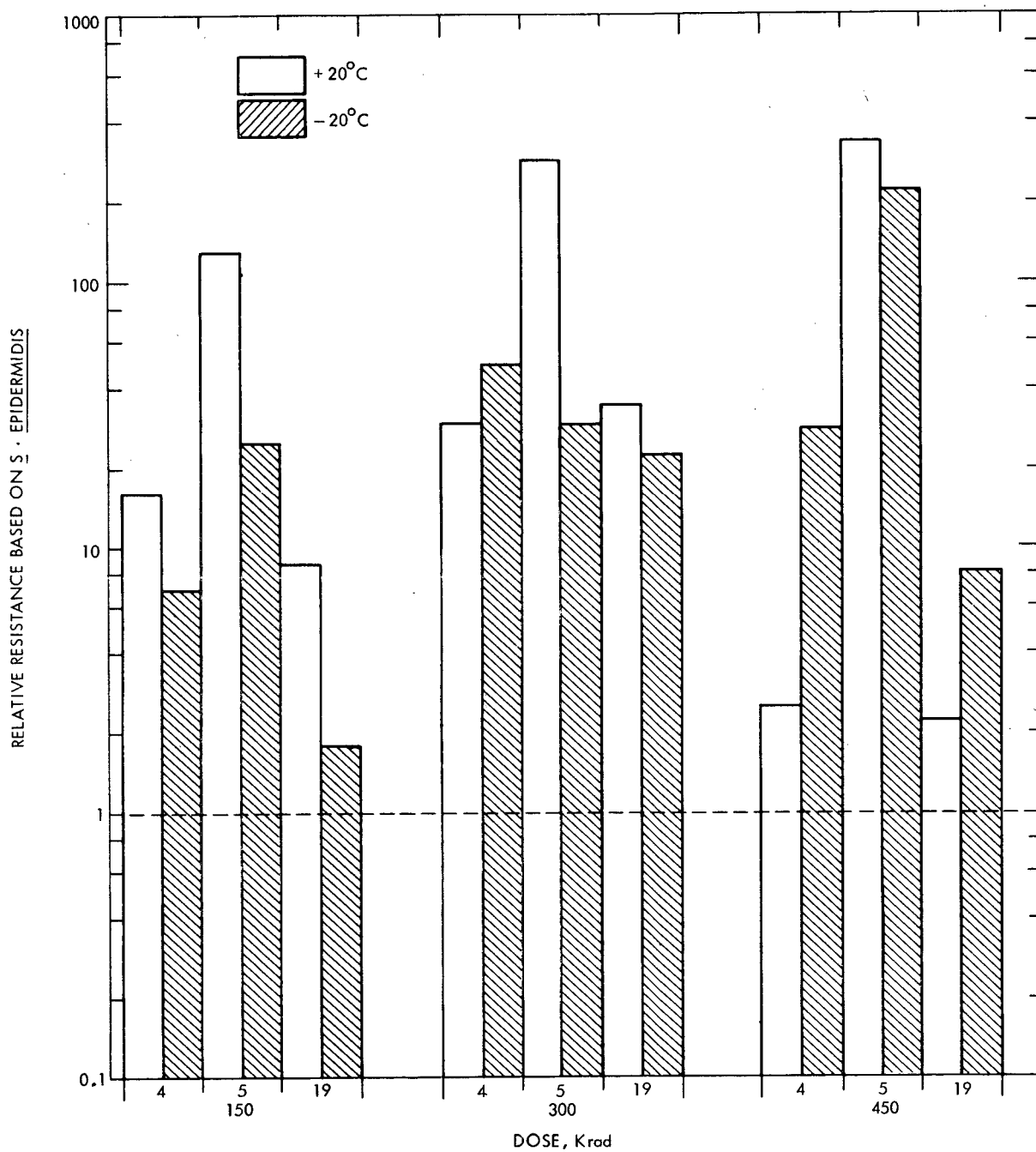


Fig. 3-9. Relative resistance of nonsporeforming isolates to 2 MeV electrons at different doses

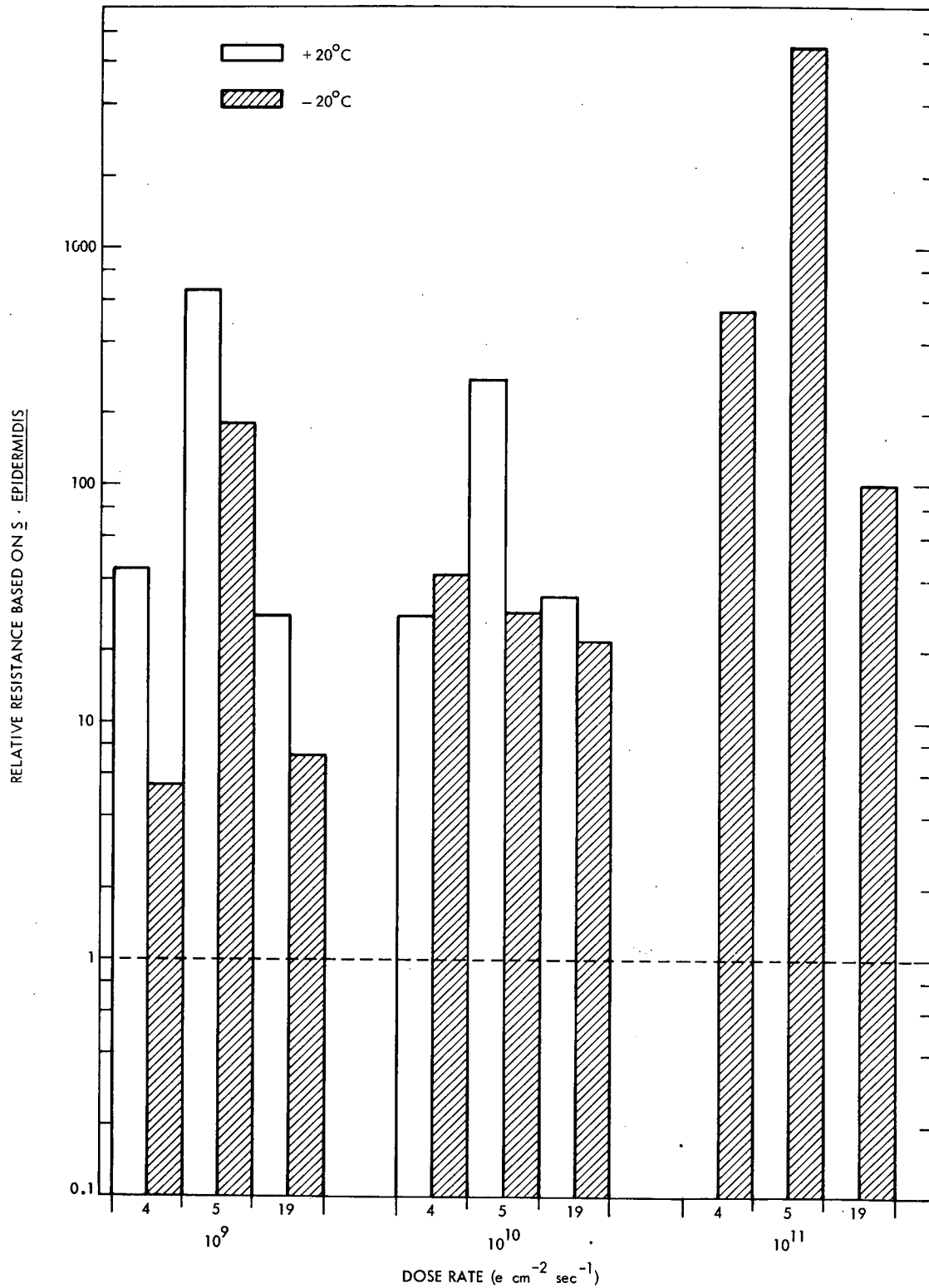


Fig. 3-10. Relative resistance of nonsporeforming isolates to 2 MeV electrons at different doses

Table 3-15. Relative resistance of nonsporeforming isolate
(No. 5) to 2 MeV electrons^a

Dose, Krad ^b	Temperature		Dose Rate, e cm ⁻² sec ^{-1c}	Temperature	
	20°C	-20°C		20°C	-20°C
150	5.16	2.08	10 ⁹	6.76	11.00
300	6.14	0.54	10 ¹⁰	6.14	0.54
450	21.50	7.76	10 ¹¹	15.70	9.26

^aRelative resistance as compared to B. subtilis var. niger.
^bVariable dose with constant dose rate at 10¹⁰ e cm⁻² sec⁻¹.
^cVariable dose rate with constant dose at 300 Krad.

Siegel, in a recent article (1) states, "Space Bioscience is itself a collection of disciplines built upon a highly interdisciplinary base. As in any other area of scientific endeavors, the major effect is concerned with norms..." He concludes this thought with, "We believe that the study of biological performance under acute stress generated by extreme environments will reveal basic properties of living matter that cannot be seen under more or less 'normal' circumstances..."

Siegel's remarks serve to introduce the area of the probability of growth because when the maximum and minimum limits of temperature, moisture, pressure, and other environmental factors are considered as a basis for life processes, many of the examples cited appear bizarre. It is very possible that this appearance is based upon too few experiments - usually of a serendipic nature - performed to reveal these limits.

This discussion will restrict itself to those severe environments that allow the growth of a microorganism and will not include conditions that allow only survival which, in most instances, are of a more severe nature.

Table 3-16 presents referenced data of the maximum and minimum limits of particular environmental factors permitting growth.

Table 3-16. Limits of environmental factors governing microbial growth

Environmental Factors	Minimum/Maximum Limit	Literature Cited
Moisture, A_w	0.11	3
	0.999	4
Temperature, °C	-34, -18	6, 5
	93, 104	7, 8
Pressure		
Hydrostatic, atm	1	11
	1000	11
Barometric, atm	0.01	12, 13
	10.5	14
Atmospheric composition	100% CO_2	12, 13, 15
	100% O_2	14
	5% NH_3 , 45% H_2 , 50% CH_4	16
	15% NH_3 , 35% H_2 , 50% CH_4	16
	15% NH_3 , 75% air	16
	50% NH_3 , 50% CH_4	17
	25% NH_3 , 25% CH_4 , 50% air	17

E

3.2.4.1 Moisture. Perhaps the chief constraint on the growth of microorganisms is the availability of moisture. From a biological point of view the availability of moisture has been described as that amount of moisture, or water, in the environment that is available for use by the organism. The biologically available moisture, termed water activity (A_w), is related to the vapor pressure (P) of the environment at a given temperature by the following equation:

$$A_w = P/P_o \quad (1)$$

where P_o is the vapor pressure of pure water at an equivalent temperature. A_w is also related to the equilibrium relative humidity (ERH) of the environment as follows:

$$A_w = \frac{\text{ERH}}{100} \quad (2)$$

The A_w concept was thoroughly discussed by Scott (2) and is an interesting concept since temperature, osmotic pressure, and other factors that affect vapor pressure of an environment at the same time affect its A_w . Scott found that growth of a number of bacterial species would not occur below an A_w of 0.82 which corresponds to a temperature in supercooled media of about -20°C . The lower limit for mold growth is an A_w of 0.70 corresponding to a temperature of -35°C .

Siegel and Roberts (3) reporting on the microbiology of saturated salt solutions found a salt-dependent bacterium that grew in saturated lithium chloride nutrient broth whose A_w was 0.11. With the recent work of Favero et al (4) reporting the growth of Pseudomonas aeruginosa in distilled water of hospital mist therapy units, the maximum and minimum limits of A_w permitting growth of microorganisms would be 0.999 and 0.11.

3.2.4.2 Temperature. Borgstrom (5) reported the growth of many mold and yeast species in concentrated fruit juice and sugar solutions at -18°C . An excellent review article by Packer et al (6) cited the work of McCormack who

isolated a pink yeast capable of growing at -34°C . In this same review article, the common occurrence of many species of bacteria, molds, and yeast growing at -5°C to -7°C was evident.

The lower limit of temperature permitting growth of microorganisms is perhaps dependent upon two factors: 1) temperature as related to enzyme kinetics; and 2) temperature as related to available water.

The present maximum temperature permitting growth of microorganisms was established by the continuing work of Brock et al (7) which deals with the microbial ecology of hot springs. This recent paper describes the significant uptake of radiolabeled substrates at 93°C (boiling at the altitude of the hot spring) while significant uptake did not occur at 97°C (superheated).

Hydrostatic pressure can influence the maximum temperature for growth and enzyme activity. ZoBell (8) reported the growth of sulfate reducing marine bacteria at 104°C when accompanied by a hydrostatic pressure of 1000 atm. Eyring et al (9) found that hydrostatic pressure up to 680 atm increased yeast invertase activity at 40°C and Johnson et al (10) reported that the thermal inactivation of tobacco mosaic virus protein at 72°C was retarded by hydrostatic pressures up to 680 atm.

Thus, the lowest temperature permitting growth of a microorganism is in the neighborhood of -30°C while the highest temperature permitting growth is about 100°C .

3.2.4.3 Pressure. Maximum and minimum values for two types of pressure are considered: hydrostatic pressure and barometric pressure.

1. Hydrostatic. The Galathea expedition over the years 1950-1952 brought up deep core samples of marine sediment (11). Samples taken from the Philippine Trench, representing water depths between 10,120 m to 10,210 m with associated hydrostatic pressures higher than 1000 atm (the pressure depth gradient in the sea is approximately 0.1 atm per meter), had viable bacteria ranging from 10^3 to 10^6 organisms per gram of wet mud. Some of the bacteria were obligate barophiles and required 600 to 1000 atm for growth.

The limits for hydrostatic pressure can be set at 1 and 1000 atm.

2. Barometric. The minimum barometric pressures demonstrating growth of microorganisms were the Martian simulation studies reported by Hawrylewicz et al (12 and 13). Spores of Bacillus cereus germinated with out-growth and subsequent growth in an Earth atmosphere at 10 torr (12) and growth of Staphylococcus aureus occurred in Earth atmosphere as well as an atmosphere of carbon dioxide at 10 torr (13).

Studies into the effects of elevated barometric pressure have chiefly reported the toxic effect of gases like oxygen at hyperbaric levels on microorganisms. Growth in the environment was generally not determined because the interest was directed towards whether the hyperbaric atmosphere exerted any toxic effect extending beyond exposure.

However, the studies of Caldwell (14) demonstrated growth of Escherichia coli, Staphylococcus albus, Staphylococcus aureus, Pseudomonas pyocyaneus, Aspergillus niger, and Mucor hiemalis in hyperbaric air at 10.5 atm.

The barometric range permitting growth of microorganisms appears to be approximately 0.01 to 10.5 atm.

3.2.4.4 Gaseous Environment. In close association with maximum and minimum limits of environmental parameters like temperature and pressure the possibility of microorganisms growing in gaseous environments other than air (Earth atmosphere) should also be considered.

The previously cited Martian simulation (12 and 13) and hyperbaric studies (14) demonstrated growth of species of bacteria and molds in 100% CO₂ and 100% O₂ atmospheres, respectively. The reported studies with the alga, Cyanidium caldarium, in 100% CO₂ at 1 atm showed that after an adaptation period of several days the growth rate and packed cell volume of the CO₂ grown cells exceeded that of the air grown control group (15).

The severe environment studies of Siegel and Giumarro (16) showed that microorganisms grew on specimens of Euphorbia and other xerophytes during 2 months in ammonia-methane, ammonia-hydrogen, and ammonia-air atmospheres. In spite of the toxicity of ammonia a variety of bacteria, molds, and yeast appeared to proliferate in these environments.

Additional studies by Siegel and Giumarro (17) demonstrated the growth of a bacterial species, Kakabekia umbellata Barghoorn, in atmospheres of 50% ammonia-50% methane and ammonia-air (39% N₂, 10% O₂, 25% CH₄, 25% NH₃, 1% other, including CO₂).

There are two biological events that need be considered in future missions to the outer planets: 1) the possibility of life forms existing in a planetary atmosphere like Jupiter; and 2) the possibility of Earth life forms growing in a planetary atmosphere like Jupiter.

The recent review article by Sagan (18) lends credence to both events. From a literature survey which includes his own studies, Sagan presents strong evidence for the existence of organic molecules on Jupiter and further, if organic molecules are present, then consideration of the existence of life forms on that planet becomes germane. He indicates that at a level approximately 50 km below the ammonia cirrus clouds water and aqueous ammonia solutions are present at temperatures approaching 300° K and pressures of the order of tens of atmospheres. He indicates that heterotrophy appears to be the simplest mode of life on a planet containing abundant organic molecules produced abiotically, but he cautions that photosynthetic autotrophy is also a distinct possibility. He stresses the requirement for performing Earth-based experiments utilizing a mixture of methane, ammonia, and water at room temperature and 1 atm pressure as a medium for microbial growth studies. The previously cited studies of Siegel and Giumarro (16, 17) indicate the distinct possibility of Earth based microorganisms surviving and growing in this type of environment.

The observation of Vallentyne (19) seems quite appropriate in conclusion, "The fact that most living species conform physiologically and ecologically to average Earth conditions should not be taken to indicate any inherent environmentally based physiochemical conservatism of living matter."

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3.2.5 Release of Microorganisms From Solid Materials

The release of microorganisms from solid materials were studies performed under JPL Contract Number 952916 by The Boeing Company, Seattle, Washington.

The following, taken from the final report, is the authors' abstract of those studies (Gustan, E.A., and Olson, R.S. "Release of Microorganisms from Solid Materials", Final Report JPL Contract No. 952916, The Boeing Co., Seattle, Washington, May 1971).

This contract consisted of three study phases that provided information on the release of microorganisms by hard impact and determined the effect of aeolian erosion on the release of microorganisms. The first phase was initiated to determine the efficiency of grinding, as compared to dissolution, for recovery of microorganisms from solids. An adjustment constant of 20 was derived from the data that can be used to equate bacterial spore counts obtained by grinding with those obtained by dissolution.

Phase II was conducted to determine the percentage of microorganisms released due to hard impact of Eccobond onto sand. This study provides additional data to JPL Contract Number 952511. In this study, Eccobond was impacted onto sand at velocities of 168, 457, 945 and 1554 m/sec. The results showed that less than 1 percent of the available organisms was released by impact.

The Phase III study was initiated to determine the percentage of bacterial spores released from methyl methacrylate and Eccobond by aeolian erosion. Sand, accelerated by air or carbon dioxide, was used to erode 0.25 grams of material from one gram discs. The results showed that less than 1 percent of the available organisms was released by the erosion process.

3.3 RELEVANCY TO PLANETARY QUARANTINE

The natural space environmental studies are directly related to Planetary Quarantine by providing data and conclusions as to how the natural space environment effects survival of microorganisms. Certain summary statements, based upon results to date, can be made: 1) spacecraft launch pressure changes cannot be considered as an agent to substantially reduce

spacecraft bioburden which, if true, could lead to relaxation of present planetary quarantine constraints; 2) current experiments, utilizing forms of radiation encountered in the natural space environment, will provide useful information that can be related to the possible reduction of spacecraft bioburden as a result of exposure to the environment; and 3) the release of microorganisms from solid materials and a previously completed study, release of microorganisms from impacted materials, established for Planetary Quarantine consideration that less than 0.1 log of the microorganisms available in the test materials were released by either impaction or aeolian erosion.

3.4 PROBLEM AREAS

With the exception of the sporeformer viable cell counts of the launch profile studies, para. 3.2.1.3, no problems were encountered.

3.5 FUTURE ACTIVITIES

3.5.1 Effect of Simulated Spacecraft Launch Pressure Changes on Survival of Microorganisms

With the exception of experiments suggested in para. 3.2.1.3 of this report no future activities are planned. These experiments were suggested in an attempt to find out why, with particular isolates, the viable cell counts after exposure to the test conditions were higher than viable cell counts of populations initially present when the test conditions did not permit proliferation. Whether this was a result of the test environment or recovery medium is not known at this time.

3.5.2 Effect of Ultra-High Vacuum and Heat on the Survival of Microorganisms

Experiments will be performed to determine the combined effects of long term exposure to space vacuum and temperature on the survival of microorganisms isolated from MM '71 spacecraft. The fourteen organisms previously used in the launch profile studies and currently being used in the radiation studies will be tested for their resistance to space vacuum and temperature. A test fixture has been designed to permit the testing of the fourteen organisms at four temperatures (-40°C, 25°C, 40°C and 55°C). After various

exposure times, up to 180 days, microbiological assays for survivors will be conducted.

3.5.3 Effect of Space Radiation on Survival of Microorganisms

The future activities of the space radiation studies will be concerned with the analysis of data from the 12 and 25 MeV electron tests; the analysis of data from the 3 keV and 2 MeV proton tests; and the scheduling and completion of the 12 and 25 MeV proton test matrixes which will also include data analysis.

3.5.4 Release of Microorganisms from Solid Materials

No future activities planned.

3.5.5 Effect of Space Ultraviolet Irradiation on Survival of Microorganisms

Future activities will involve determining the effect of ultraviolet irradiation in a space vacuum on the survival of microorganisms isolated from MM '71 spacecraft. Preliminary discussions have considered possible dose rates that will be achieved from different spacecraft missions to Mars or Jupiter, equipment requirements for the experiments, and the initiation of a literature survey.

3.5.6 Probability of Growth

Future activities will involve the development of possible analytical approaches to utilize existing knowledge of maximum and minimum limits of terrestrial environmental parameters for bacterial growth and predict whether or not particular regions of a planetary environment could support growth of terrestrial bacteria or life forms.

3.6 PRESENTATIONS

Knittel, M.D., Favero, M.F., and Green, R.H. "Microbiological Examination of Electrical Cable from Surveyor III", Presented at Second Annual Lunar Conference, Houston, Texas, January, 1971.

Knittel, M.D., Godfrey, J.F., Hagen, C.A. and Taylor, D.M.
 "Survival of Spores and Nonspore Forming Bacteria During Simulation of a
 Spacecraft Launch Pressure Profile". Presented at American Society for
 Microbiology Annual Meeting, Minneapolis, Minn. May, 1971.

The following presentations were made at the semi-annual NASA
 Sterilization Technology Seminar, Seattle, Washington, June, 1971.

Hagen, C.A., "Effect of Vacuum Profile on Survival of
 Microorganisms".

Olsen, R.L., "Release of Microorganisms from Solid Materials".

Taylor, D.M. "Effects of Space Radiation on Survival of
 Microorganisms".

The following presentations were made at the annual Committee on
 Space Research meeting, Seattle, Washington, June, 1971.

Gustan, E.A., "Effects of Aeolian Erosion on Microbial Release
 from Solids".

Taylor, D.M., "A Re-Evaluation of Material Effects on Microbial
 Release from Solids".

3.7 PUBLICATIONS

Hagen, C.A., Godfrey, J.F., and Green, R.H., "The Effect of
 Temperature on the Survival of Microorganisms in a Deep Space
 Vacuum". Accepted for publication in Space Life Sciences, 1971.

SECTION IV

PLANETARY QUARANTINE SUPPORTING ACTIVITIES

NASA No. 191-58-28-02-55

Cognizance: D. M. Taylor

Associate Personnel (AVCO Corp.): C. Hagen, G. Renninger

G. Simko, C. Smith

4.1 INTRODUCTION

Planetary Quarantine Support Activities include the technical assistance for all Space Research and Technology (SR&T) tasks and the operation and maintenance of the microbiological laboratories at JPL. This is accomplished through a contract with AVCO Corporation, which at the present time, employs individuals constituting a multi-disciplinary group of contamination control engineers, microbiologists, physicist, statistician-computer scientist, and associated support personnel.

4.2 SIGNIFICANT ACCOMPLISHMENTS

In addition to the technical support provided to other SR&T tasks, personnel of the Planetary Quarantine Laboratory were involved with the following tasks:

- 1) The isolation and classification of bacteria recovered from MM '71 spacecraft for possible future use in studies involved with determining bacterial resistance to space environmental parameters like vacuum and different types of radiation.
- 2) The evaluation of a possible relationship existing between non-viable particle size and bioburden.
- 3) Environmental monitoring for the presence of molds in spacecraft assembly and test areas at JPL and AFETR.

4.2.1 General Support

Some of the tasks, although reported elsewhere in this document, are listed in this section since the associated personnel are on virtual full time assignments to these tasks.

4.2.1.1 Spacecraft and Monitoring Methods and Procedure (NASA Task No. 191-58-63-03-55). A contamination control engineer and two microbiologists are involved with the refinement of techniques and procedures necessary to estimate the microbiological burden on spacecraft, the bioassay storage and data retrieval system, and the documentation and certification procedures for verification and environmental parameters in laminar airflow and nonlaminar airflow facilities. The contamination control engineer and one microbiologist performed similar tasks at AFETR for approximately 3 months prior to MM '71 spacecraft launch.

4.2.1.2 Natural Space Environmental Studies (NASA Task No. 191-58-62-04-55). Four microbiologists and one vacuum engineer are involved with determining the effect of simulated spacecraft launch pressure changes on the survival of bacteria isolated from MM '71 spacecraft; determining the effect of space radiation, electrons and protons at different energies, fluxes, and doses, on the survival of bacteria isolated from MM '71 spacecraft; and providing assistance for the design, construction, and operation of vacuum equipment required for the above studies.

4.2.1.3 Thermal Resistance of Microbes in Hardware Assembly Areas (NASA Task No. 191-58-61-06-55). This task is concerned with determining the dry heat resistance at 125°C in both air and nitrogen atmospheres of spores isolated from MM '71 spacecraft and associated assembly and test areas at JPL and AFETR.

4.2.1.4 Post Launch Recontamination Studies (NASA Task No. 191-58-63-11-55). A physicist is involved with determining the effect of flight environments on vehicle burden redistribution.

4.2.1.5 Microbial Burden Prediction Model (NASA Task No. 191-58-63-06-55). A statistician-computer scientist is involved with development of a computer

code for predicting microbial burden on spacecraft surfaces during system assembly and test procedures.

4.2.2 Isolation of Bacteria From MM '71 Spacecraft

The previous Semi-Annual Review reported the procedures used for the isolation and identification of bacterial isolates recovered from four stage burden microbiological assays.

Bacterial isolates were recovered from a subsequent stage burden microbiological assay at AFETR. The isolates were tentatively grouped according to source, i. e., isolates recovered from solar panel front, shroud, and remainder of spacecraft surfaces sampled at the encapsulation assay. The isolates are being retained for future identification and possible experiments related to the Natural Space Environmental Studies.

4.2.3 Relationships Between Nonviable Particles and Bio-Burden

The assay procedures, using membrane filters, were described in the previous Semi-Annual Review. The procedures were conducted in spacecraft assembly and test areas at JPL and AFETR. The data are being statistically analyzed and will be reported upon at a later date.

4.2.4 Environmental Monitoring for Molds

The test procedure adopted for monitoring of mold fallout in spacecraft assembly and test areas was described in the previous Semi-Annual Review. At that time, data reported up to 15 January, 1971 showed a trend indicating the numbers of molds settling out from the environment were greater than the numbers of bacterial spores.

Figure 4-1 presents data from 22 January, to 14 May, the last test prior to spacecraft launch. The data appear as plots of the weekly average of molds settling per square foot together with the high and low values at a particular sampling site for that week. The curve below the x-axis represents zero mold count at a particular site; the low for the week. The weekly average of bacterial spores settling per square foot is included for comparison.

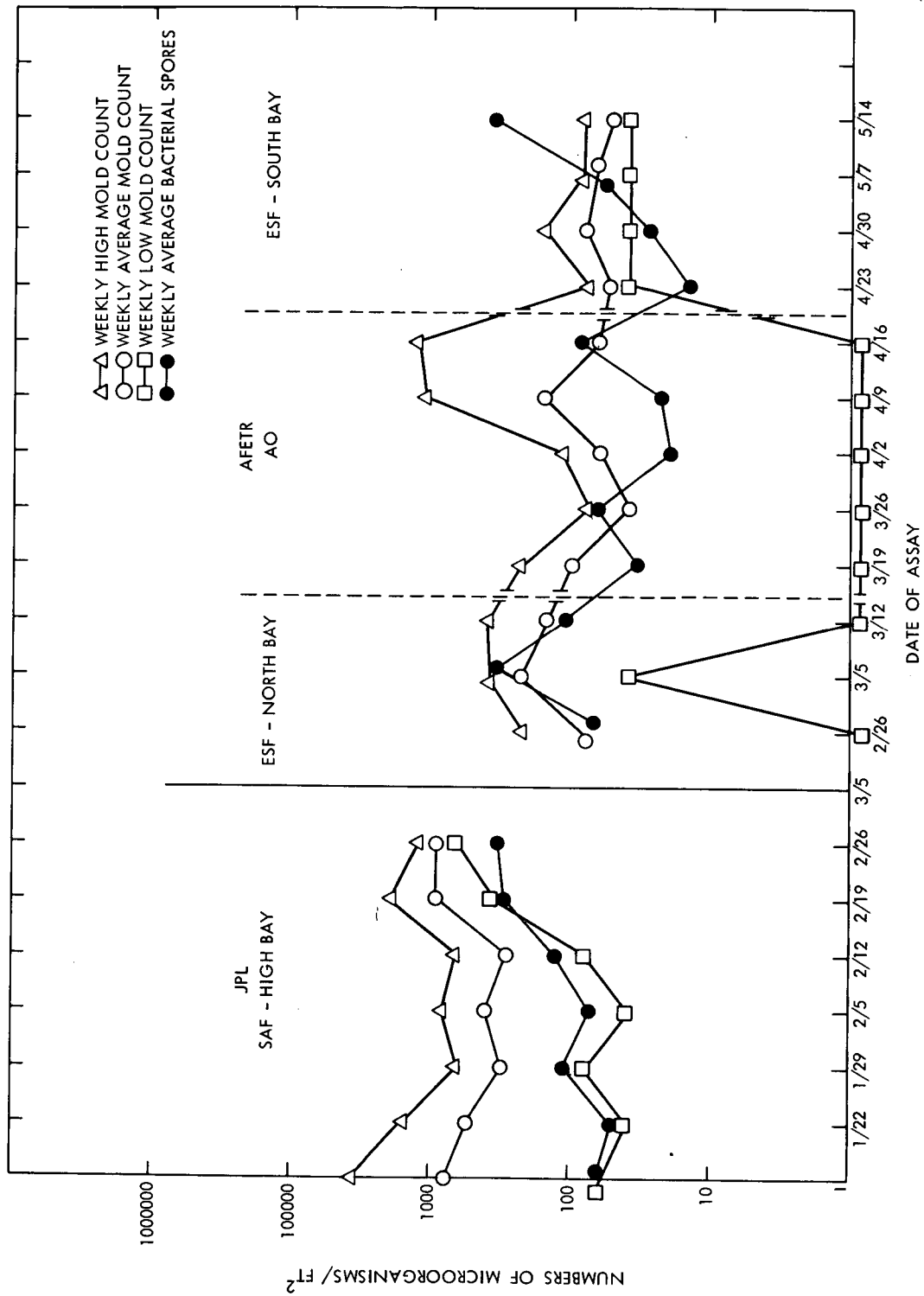


Fig. 4-1. Environmental monitoring for molds

The same general trend previously reported was evident: the weekly average of molds settling per square foot was higher than the weekly average of bacterial spores. There also appeared to be less mold and bacterial spore fallout at AFETR than at JPL though it is not known at this time whether the difference is significant.

4.3 RELEVANCE TO PLANETARY QUARANTINE

The present activities benefit planetary quarantine programs in the following manner:

- 1) The establishment of an existing relationship between nonviable particles and bio-burden would allow the standardization of a rapid method to determine spacecraft bio-burden.
- 2) The enumeration of molds, although not a part of NASA standard procedures, does raise questions related to planetary quarantine analyses that consider bio-contamination allocations and survival of molds in space environments.

4.4 PROBLEM AREAS

None

4.5 FUTURE ACTIVITIES

The continued effort of Planetary Quarantine Support Activities will be directed toward maintaining that required level of technical competency in the area of planetary quarantine. The following tasks are to be concluded:

- 1) Evaluate data to substantiate possible association of bio-burden with the size of nonviable particulate matter.
- 2) Enumerate sporulating bacteria recovered from nonheat shocked samples taken during the encapsulation microbiological assay milestone at AFETR.

4.6 PRESENTATIONS AND PUBLICATIONS

None

SECTION V

SPACECRAFT MONITORING METHOD AND PROCEDURES

NASA No. 191-58-63-03-55

Cognizance: D.M. Taylor, R.C. Koukol

5.1 INTRODUCTION

During the second semi-annual period of the fiscal year 1971 (1 Jan - 30 June), the task efforts were sequential to that reported in Document 900-484; namely, spacecraft monitoring and environmental monitoring for post-encapsulation studies at JPL facilities and the Air Force Eastern Test Range, Florida. The objectives were:

- 1) Refine the techniques and procedures necessary for the direct biological estimation of bio-burden on an assembled spacecraft or subsystem.
- 2) Provide data to update the input parameters for the microbial burden prediction model.
- 3) Provide microbial isolates for other studies.

Furthermore, the problems identified in the Planetary Quarantine Advisory Committee reports were addressed to developing criteria for:

- 1) Establishing the number of samples required for direct estimation of bio-burden on a surface.
- 2) Determining where to sample a surface.
- 3) Estimating the burden on a specific surface from individual samples.
- 4) Methods of combining estimates from a number of surfaces.

The approaches to meet these objectives and problems were as follows:

- 1) To determine the relationship of environmental cleanliness and the spacecraft bio-burden.
- 2) The development of sampling procedures for problem areas, viz: cabling, thermal blankets, solar panels, and high gain antenna.

- 3) The refinement of bio-burden estimation consisting of number of samples taken, location of sample site and data extrapolation.

5.2 SIGNIFICANT ACCOMPLISHMENTS

5.2.1 Relationship of Environmental Cleanliness Level and Spacecraft Bio-Burden

This activity was continued for data collection pertaining to the environmental sampling and spacecraft monitoring for STAGE VII Pre-Encapsulation and STAGE VIII Decapsulation.

5.2.1.1 Environmental Monitoring. The methods employed for data gathering in Document 900-484 were continued. The data obtained is shown in the figures described below.

- 1) Figures 5-1 and 5-2 depict the mean particle levels from Royco Sampling in the SAF High Bay-Hanger AO and the SAF Tent Area, respectively.
- 2) Figures 5-3 and 5-4 depict the mean particle levels from Reynier Sampling in the SAF High Bay-Explosive Safe Facility and the SAF Tent Area, respectively.
- 3) Figures 5-5 and 5-6 depict both the viable and heat shocked particle levels from the settling strips in the SAF High Bay-Explosive Safe Facility and the SAF Tent Area-Explosive Safe Facility, respectively.

The particulate data obtained for the STAGE VII and STAGE VIII phases are observed to be within prescribed limits: Class 100,000 for the SAF High Bay-Explosive Safe Facility High Bay; Class 10,000 for the Hangar AO; and Class 100 for the tents in both SAF and Explosive Safe Facility. The viable counts recorded show a correlation respective to the particulate data obtained from the fallout strips.

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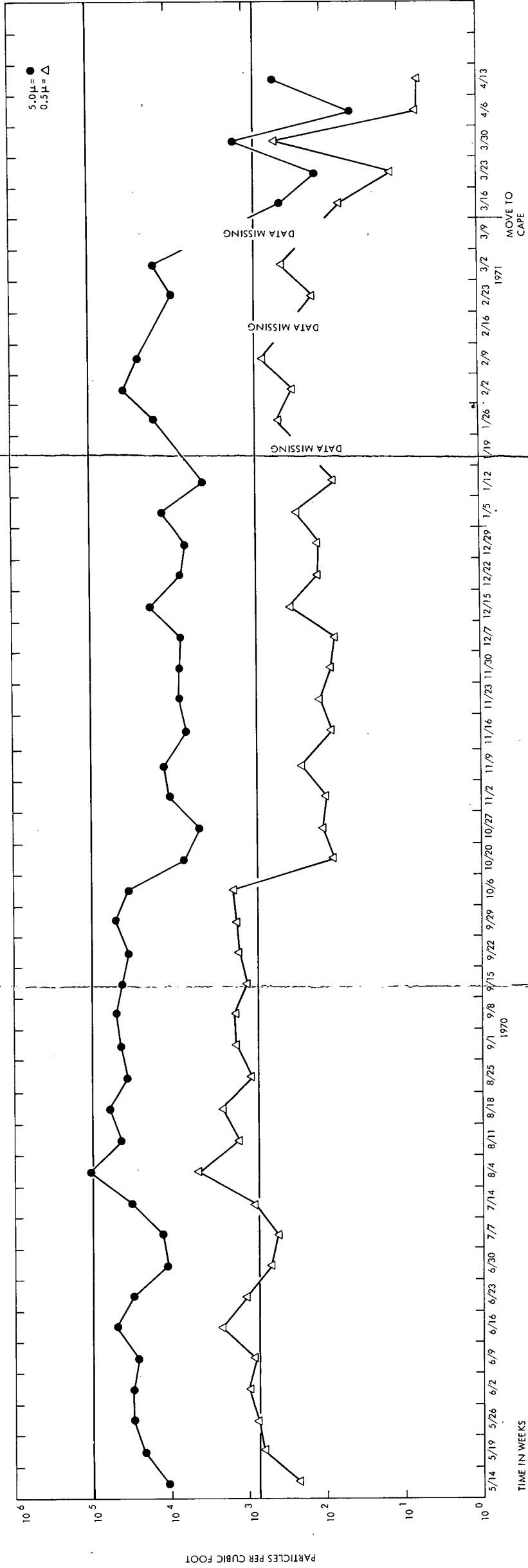


Fig. 5-1

Mean particle levels in SAF High Bay

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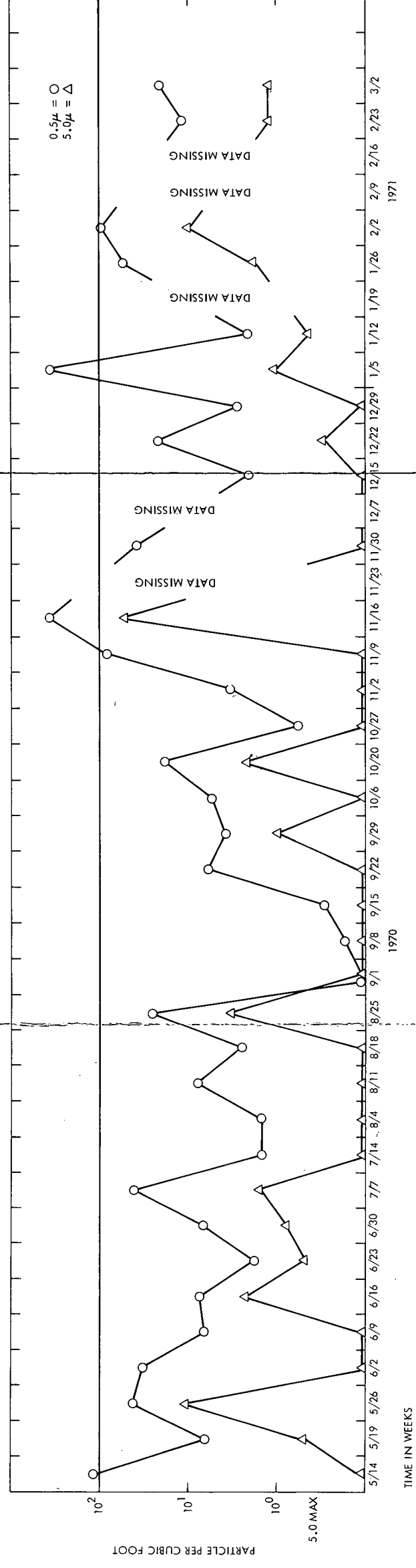


Fig. 5-2
Mean particle levels in SAF tent

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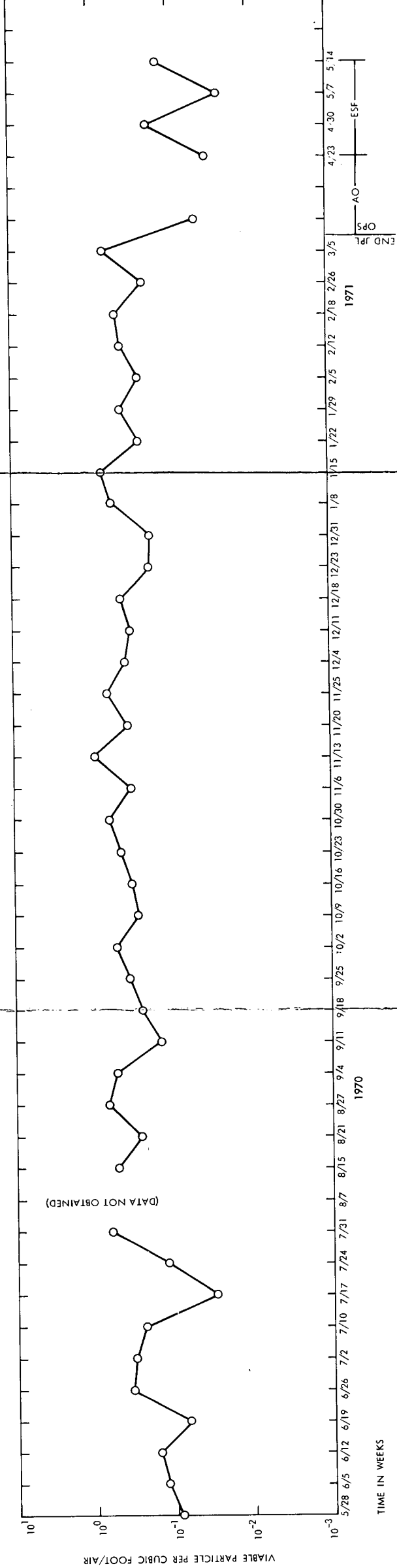


Fig. 5-3
Viable particles/ft³ of air/week at High Bay (Reyneir)

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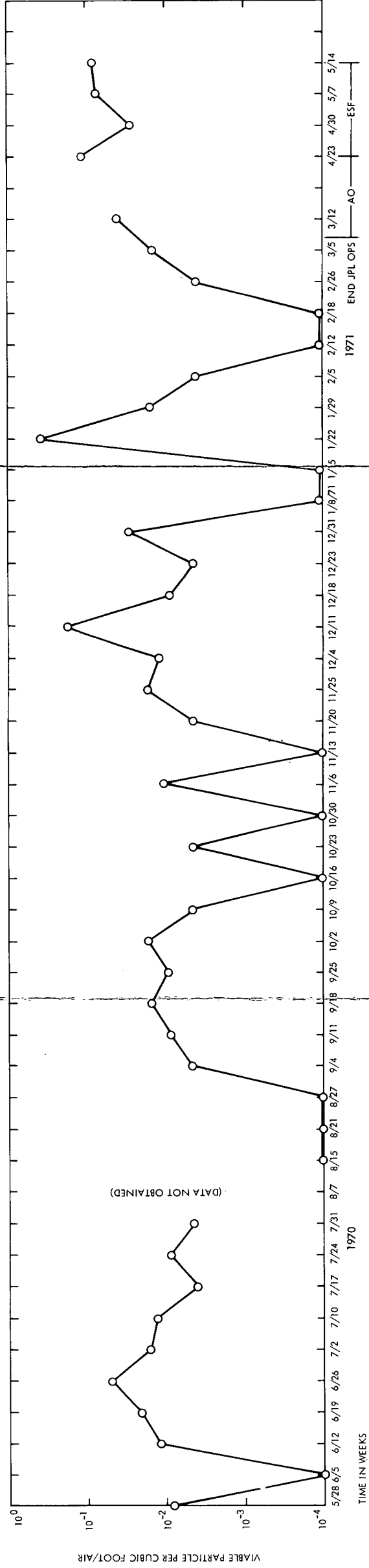


Fig. 5-4

Viable particle/1 ft³ of air/week in Tent (Reyneir)

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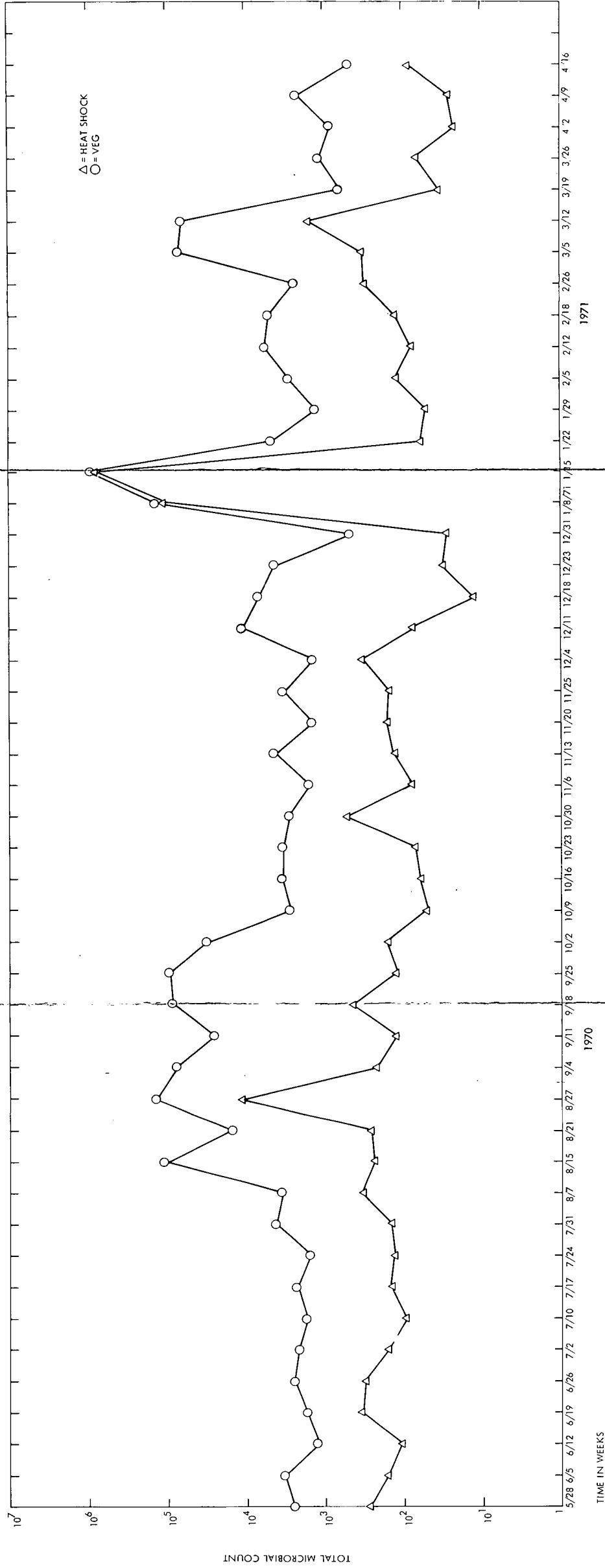


Fig. 5-5
Total count/week in SAF High Bay
5-11

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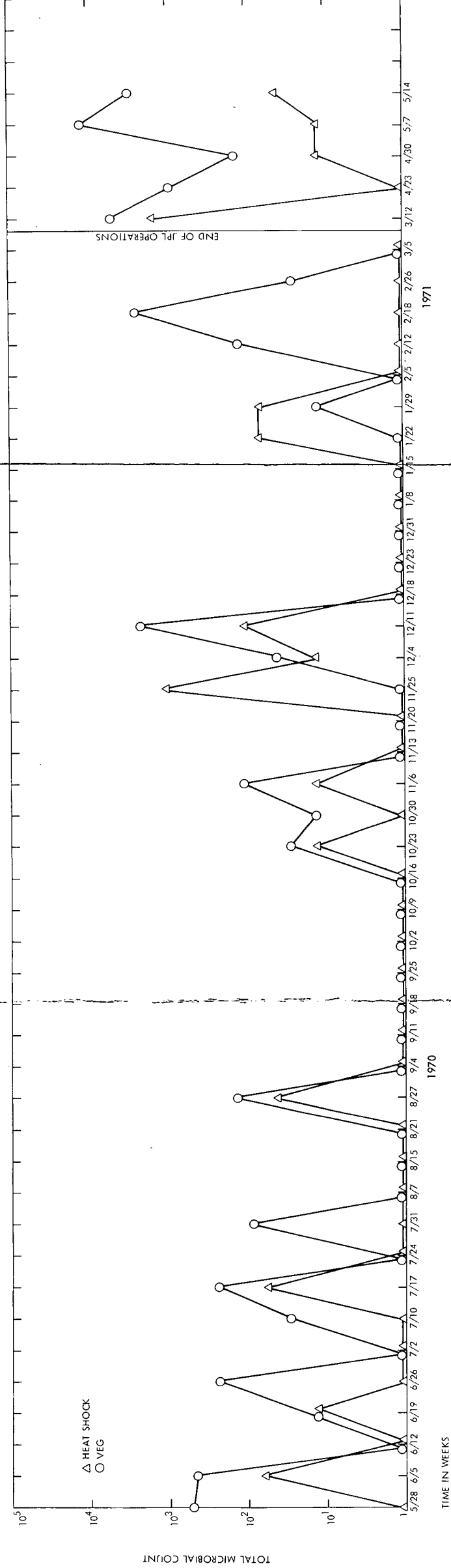


Fig. 5-6

Total count/week at SAF Tent

G

5.2.1.2 MM '71 PTM Monitoring. This phase was conducted to develop data of the respective bio-burden levels for the 8 stages indicated in Document 900-484. Specifically, during this reported period (1 Jan - 30 June, 1971), data was obtained for STAGES VII (Pre-Encapsulation) and VIII (Decapsulation). Figure 5-7 shows the bio-burden assay data for all stages (I thru VIII). An evaluation of this data will be assessed for the relationship of environmental cleanliness and spacecraft bio-burden. In addition, the sequence of activities related to the hardware handling for shipment offered an opportunity to gather data respective to pre-shipment and post-shipment. Assays were taken on items of equipment in an ambient environment during shipment and an inert environment (N_2) during shipment. Data is presented in Figs. 5-8 and 5-9. A relative significance is noted due to the variance of environments. A 1-log decrease of vegetative cells is recorded. Both reductions are attributed to a natural die-off.

5.3 PROBLEM AREAS

The interconnecting cabling, as indicated in Document 900-484, remains an unsolved problem. Due to the time limitations, no effort was undertaken toward resolving this problem.

5.4 FUTURE ACTIVITIES

5.4.1 Determining Where to Sample a Surface

The data obtained for each planned milestone (STAGES I thru VIII) will be assessed considering the surface nodosity, component geometry, and environmental aerodynamics.

5.4.2 Data Extrapolation

Interface with the task "Microbial Burden Prediction Model", shall provide statistical studies for determining:

- 1) The relationship between micro-organisms and specific types of hardware surfaces.
- 2) The distribution of spore and non-spore isolates.

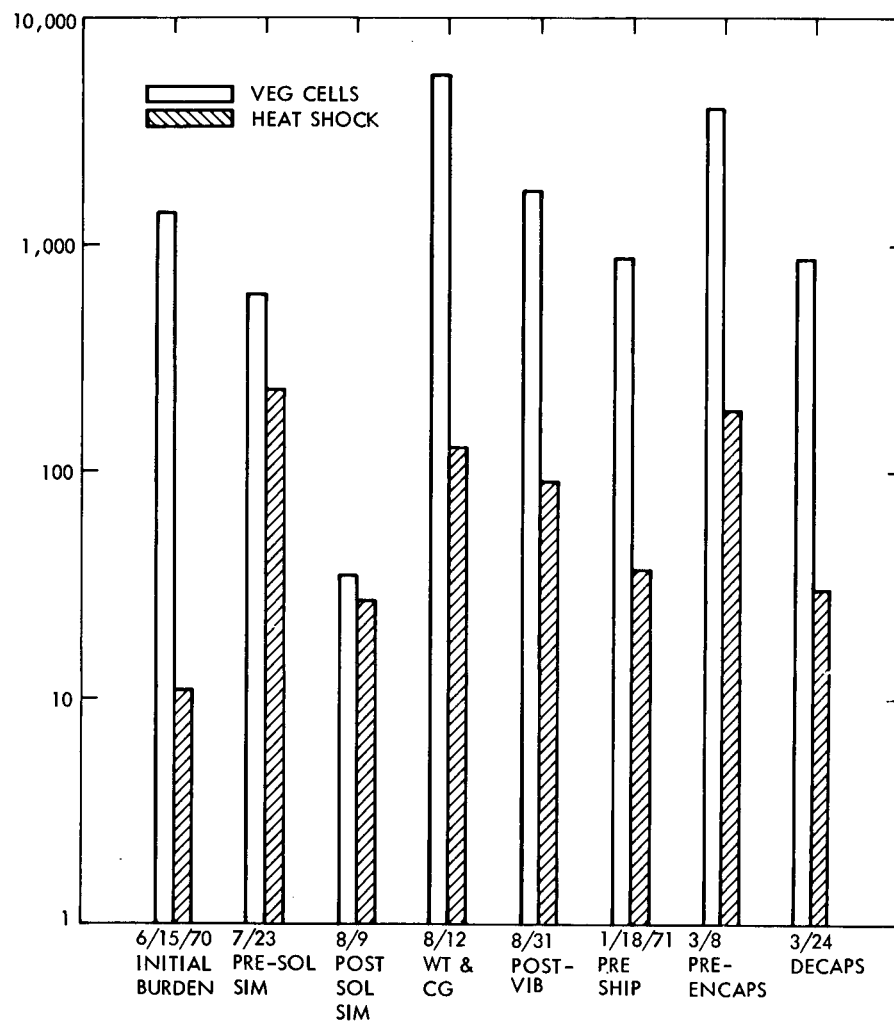


Fig. 5-7. Bio-burden assay data for all stages

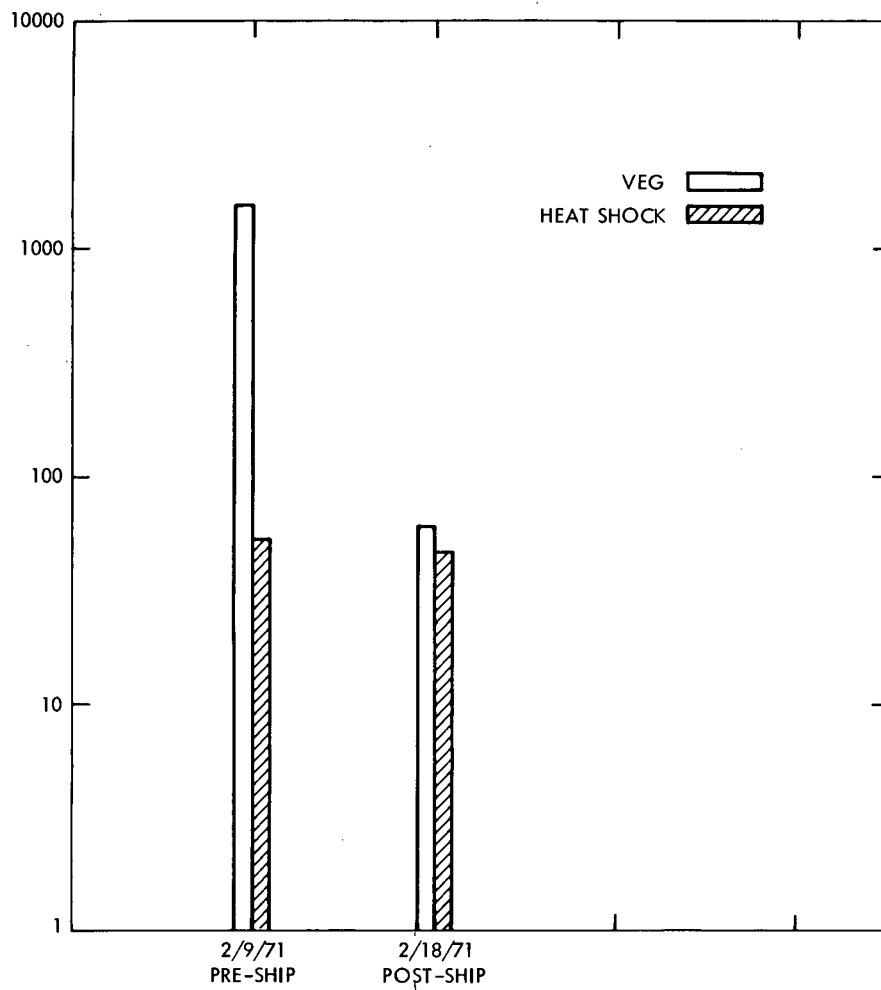


Fig. 5-8. Comparison of pre and post shipment assays, ambient environment

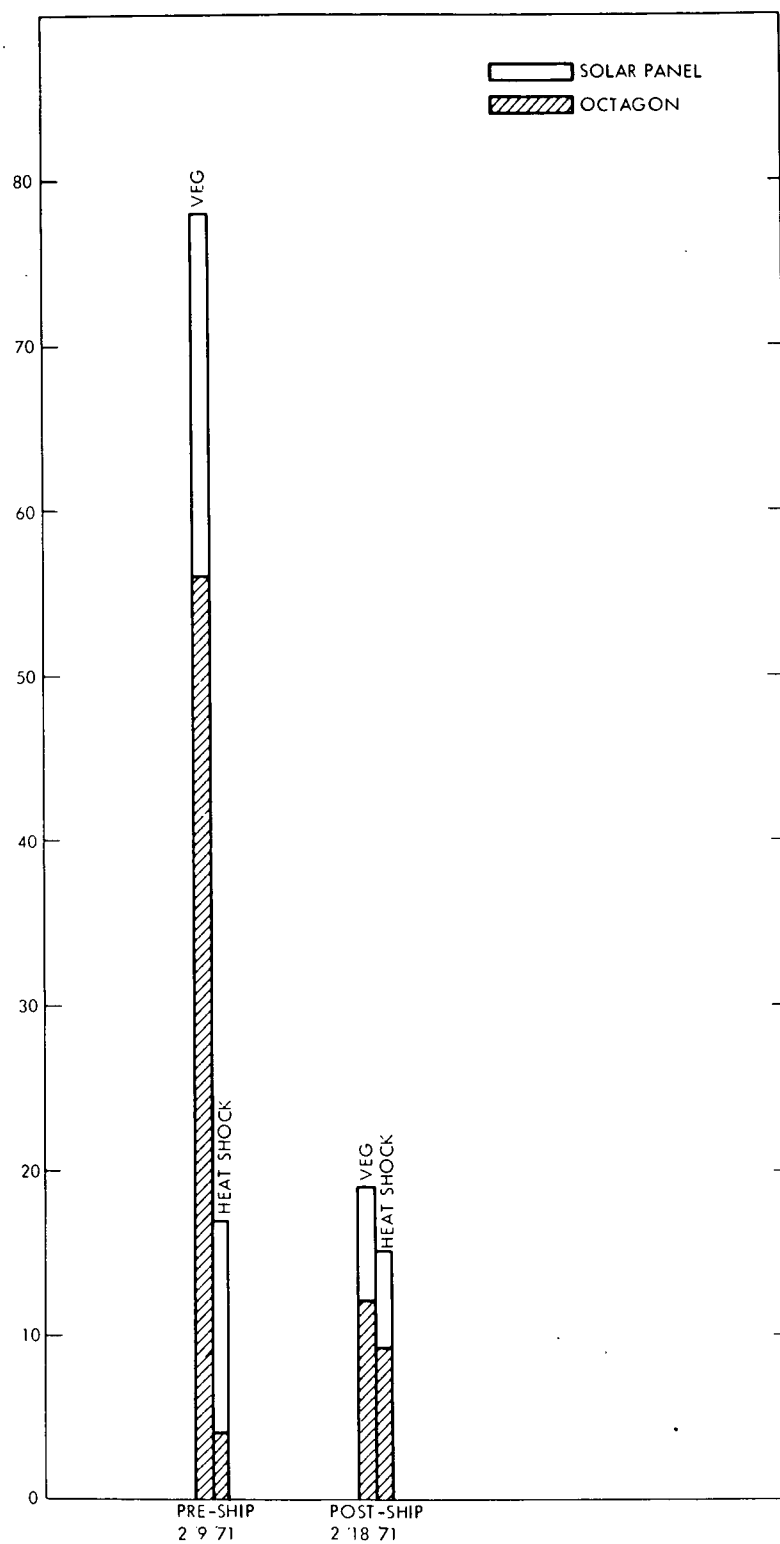


Fig. 5-9. Comparison of pre and post shipment assays, inert environment (N_2)

- 3) The effect on the total burden through varying the number of samples taken on a given piece of hardware.

5.5 PUBLICATIONS

None

SECTION VI

MICROBIAL BURDEN PREDICTION MODEL

NASA No. 191-58-63-06-55

Cognizance: A.R. Hoffman

Associate Personnel: D. Winterburn (AVCO), R. Koukol

6.1 INTRODUCTION

The Microbial Burden Prediction Model (MBPM) and its associated computer program have been developed as tools for: 1) supplementing the biological assays of a spacecraft by simulating the microbial accumulation during periods when assays are not taken; 2) reducing the number of biological assays that are required; and 3) predicting the microbial loading on the lander prior to sterilization and the other non-lander spacecraft equipment prior to launch.

When this effort was begun over two years ago, a model was needed that would permit a priori analysis of the manner in which a spacecraft was assembled in order that biological monitoring could be planned and scheduled, and optimum times for sampling determined. Also, the model could be used to optimize the assembly flow for maintaining the microbial burden at an acceptable level. During the actual assembly, the model can be used as a quality assurance control tool to ensure that the microbial burden is not becoming excessive on a given piece of hardware. Finally, a reasonable and meaningful estimate is needed at the time of encapsulation for an orbiter to be used in the post-launch planetary quarantine analysis. The prediction model, validated and verified on a continuing basis by direct assay methods, can provide such an estimate.

Early in the development of the MBPM, it was necessary to choose a method for dealing with several random variables in the model. With a desire to obtain a complete view of the distributions of resultant variables while using minimum computer run time, the decision was made to represent these variables as discrete probability density functions, or histograms. Since the details of histogram manipulation had not been fully developed, resulting difficulties and limitations were not completely understood (such as distributions

concentrating in the highest valued histogram interval). The Phase IX of the Math Model contract with Martin Marietta refined the histogram method of combining random variables to obtain more realistic predictions of the microbial burden on spacecraft.

6.2 SIGNIFICANT ACCOMPLISHMENTS

During this reporting period, the Mariner '72-2 actual assembly and test sequence emulation was completed. The assembly sequence of a typical solar panel was also emulated: it was considered important to track a typical solar panel because the combined surface area of the solar panels represents 60% of the entire spacecraft exposed area. Because predicted results for the solar panel exhibited wide fluctuations not fully understood, further study has been initiated.

Environmental microbiological data was assembled and used to construct new input histograms, and assembly operations were modified to reflect the true operations. These environmental histograms describe the fallout, or background contamination, consisting of spore and nonspore forming organisms in the different environments the spacecraft is subjected to during its assembly (JPL: Tent, High Bay, Vibration Area (Out of Tent), Simulator, Acoustic Lab; ETR: Hanger AO, Explosive Safe Facility (In Tent), Encapsulation).

The computer program was used to obtain predicted burdens on exposed surfaces for the Mariner '71-2 actual assembly and test emulation from Stage 6 until launch. A graph of this burden is shown in Fig. 6-1.

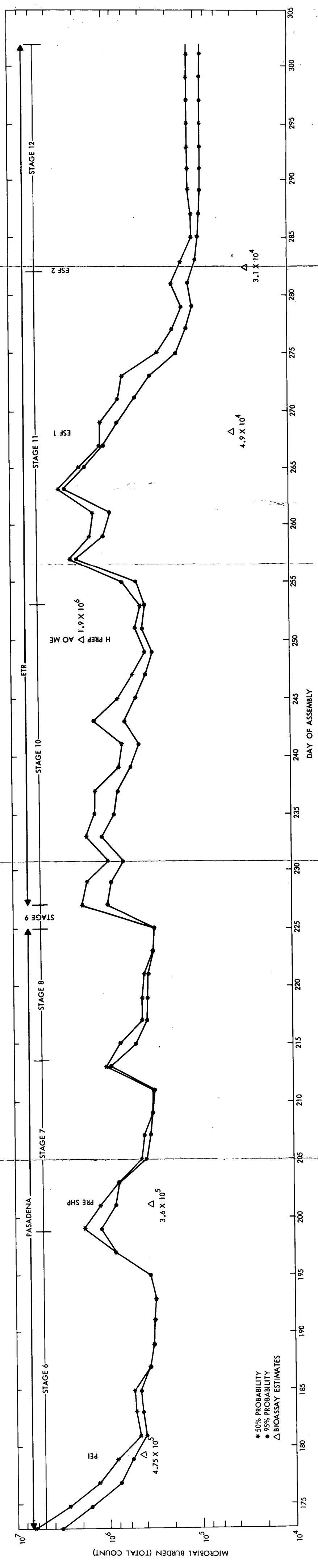
Enumeration of the microorganisms on the surface of a spacecraft is necessary for sterilization process determination of planetary landers and for preparation of probability estimates for the planetary quarantine prelaunch analysis. The methods developed and results obtained show the usefulness and necessity of the model in predicting this microbial burden, and that from any microbial accretion process in general. The model thus lends itself to related work in water and air pollution, sewage treatment monitoring and control, and work in similar fields.

FOLDOUT FRAME 2

FOLDOUT FRAME 3

900-556

FOLDOUT FRAME 1



6-3

Fig. 6-1
Microbial burden prediction model of Mariner Mars '71 Flight 2 S/C;
exposed surfaces, total count

6-3

900-556

6.3 PROBLEM AREAS

Because of the vast excursions of the predicted solar panel burdens, investigation is being made into properties of the model which could result in such variations when only one part is affected, e.g., the ways the model attributes burden accumulation to operations, environments, operation times and cleanings.

6.4 FUTURE ACTIVITIES

Tasks proposed for the future include:

- 1) Comparison of predicted burdens with those of spacecraft direct assay estimates.
- 2) Comparison of results using both histograms based on Mariner '67 data and current data.
- 3) Comparison of results of the old program to those of the new program (using current data).

Sensitivity studies similar to those performed on the Mariner '67 actual sequence and the Mariner '71 planned sequence should also be performed to provide data which will allow analyses and a better understanding of contamination processes.

SECTION VII

PLANETARY QUARANTINE CONSTRAINTS FOR ADVANCED MISSIONS

NASA No. 191-58-63-10-55

Cognizance: C.C. Gonzalez

Associate Personnel: W. Stavro

7.1 INTRODUCTION

The objectives of this task are to perform analyses necessary to define planetary quarantine constraints, determine the parameters to which constraints are sensitive, the mission strategies which satisfy these constraints, and identify problem areas which will require further research.

Current models of the atmospheres of Jupiter and Saturn indicate the possible existence of regions with conditions favorable for growth of terrestrial microorganisms. To ensure that the environments of the outer planets are not altered biologically, a study was performed to identify planetary quarantine constraints and problem areas. Potential mission strategies compatible with both mission objective and planetary quarantine constraints were investigated.

Possible sources of contamination were identified and related to mission events, flight-path-control strategies, and interplanetary environments. Relevant parameters to which planetary quarantine analyses were considered most sensitive were selected and included in subsequent analyses. A preliminary allocation model was developed and analyses performed to obtain probability of contamination values for the most significant sources. Potential problem areas in need of future research were identified.

7.2 SIGNIFICANT ACCOMPLISHMENTS

7.2.1 Tasks Conducted

The Planetary Quarantine Analyses for Advanced Missions involved the identification of contamination (sources and the suballocation of probability of contamination) among the various sources. A total allocation of 7.1×10^{-5} was assumed for each planet. Since the objective of the current analysis is not a detailed study, only major contributing sources were considered.

This report will present the results to date of the study to identify contamination sources and the relative importance of each. The main sources of contamination are considered to be large impactables such as the spacecraft and launch vehicle. In order to determine whether a contamination source will result in microbiological contamination of the planet in question, the initial steps involved trajectory considerations. (The details of the trajectory and navigation analysis were presented in the previous Semi-Annual Review.)

In addition to performing the large impactable analyses, one of the tasks performed was the identification of areas for future research. These areas pertain to work needed to be performed by the Planetary Quarantine Community in order that future projects may perform a complete PQ analysis for an outer planets mission.

7.2.2 Results of Work

7.2.2.1 Analyses. The results of trajectory and navigation analyses were based on the following assumptions:

- 1) The basic characteristics of a mission can be determined once the launch opportunity, spacecraft weight, and launch vehicle are specified. The launch vehicle considered in this study is the TITAN IID/Centaur/Burner II (2300). Both 5-segment and 7-segment Titan vehicles have been considered. The increased performance of the 7-segment over the 5-segment TITAN may be used to either decrease flight time or increase injected weight. The flight time of the 1976 J-S-P opportunity, however, is constrained by geometry (specifically the Jupiter fly-by altitude) and flight times to Pluto of less than 8.7 years are unattainable. The 1977 J-S-P opportunity does not have this geometrical constraint and the 7-segment TITAN can be used to decrease flight time. Because of these basic characteristics of the '76 and '77 J-S-P missions, it was decided to consider, for the purpose of this study, a 5-segment TITAN for the '76 opportunity and the 7-segment TITAN for the '77 opportunity.

- 2) The spacecraft weight of 1450 lbs assumed for both '76 and '77 J-S-P missions was based on the spacecraft developed in the TOPS project.
- 3) In defining the separation and retro maneuvers, certain parameters were assumed. The Spacecraft-Launch Vehicle (S/C-LV) velocity and mass ratios were taken from the Mariner Mars 1969 mission. The appropriate direction parameters of the separation and retro maneuvers were taken from the proposed Viking Mars 1975 mission because Viking has a parking orbit as do the outer planet missions. Both these missions and currently proposed missions studied here are fly-bys.
- 4) The times of separation and retro were assumed to be the same as injection due to a lack of such details at this time.
- 5) The time of midcourse corrections was assumed to be as given in Fig. 7-1.
- 6) Error mapping was assumed to be a linear process.
- 7) The probability of success for correction maneuvers was assumed to be 0.97.
- 8) The optical approach measurement errors was assumed to be 6 arc-seconds.

7.2.2.2 Results. The following summary of the results of the navigation analyses also provides identification of areas for future research.

- 1) The probability of impact allocation required by the launch vehicle, assuming the same capability for separation and deflection as in current missions, is of the same order of magnitude as that for the S/C. This departs greatly from previous missions where suballocations for the launch vehicle were a fraction of total allocation.
- 2) Unbiased nominal trajectories of the Earth-Jupiter leg will violate the probability of impact constraint at Jupiter.



Fig. 7-1. Midcourse maneuver plan for J-S-P mission

- 3) In the Earth-Jupiter leg, only the injection maneuver needed to be biased. Maneuvers 1, 2, and 3 can be aimed at the desired aim point without violating planetary quarantine (PQ).
- 4) The ΔV (needed additional velocity) penalty for removing the biasing of the injection maneuver was found to be of the order 20 m/sec based on the assumptions and parameters selected for the baseline analysis.
- 5) If Jupiter encounter is achieved within the expected accuracy (accuracy of the approach guidance instruments), the planetary quarantine constraint at Saturn will be violated. This violation can be satisfied by biasing Jupiter's aim point.
- 6) The ΔV penalty for removing the required biasing of Jupiter's aim point is of the order 10 m/sec.
- 7) Most of the S/C impact allocation for the Earth-Jupiter maneuver can be given to the injection (as opposed to allocating it equally between the injection and midcourse maneuvers).
- 8) The optimal biasing strategy for the injection maneuver based on the minimum ΔV required to correct the biasing is the strategy which minimized the expected square of the magnitude of the next midcourse correction.
- 9) The optimal strategy used in biasing the Jupiter aim point was that which minimizes the required post Jupiter ΔV to remove that bias.
- 10) The preliminary analysis performed for the Saturn-Pluto leg indicates that due to the large distances and small target planetary capture radius involved, biasing of the pre-Saturn maneuver is unnecessary.
- 11) Due to the lack of available software for targeting and navigation of multi-planet gravity assist missions, the exact biasing of post-Saturn maneuvers required to satisfy a PQ constraint for Pluto was not determined. However, it is recommended that a realistic probability of contamination constraint which can be met without putting severe penalties on a mission be applied to Pluto.

- 12) It was determined that small ΔV errors in the midcourse maneuvers did not propagate rapidly due to high injection energies of the trajectories considered. Because of this property, any type of abort bottle system to be carried on board to alter the trajectory to satisfy PQ would be too large to be of practical interest.
- 13) The assumption made at the outset of the current task, that the probability of impact of Saturn by the launch vehicle is not significant, should be investigated further due to the high probability of impact of Jupiter by the launch vehicle. Note that this high probability may be decreased by increasing the launch vehicle/spacecraft separation velocity and the deflection velocity of the launch vehicle.
- 14) Due to the rather large penalties imposed by biasing, appropriate analyses should be performed to understand and eventually qualify all possible factors which help reduce S/C (or LV) burden. Some of these factors are the thermal vacuum space environment, Jupiter's radiation belts, the entry heating of spacecraft for a planet possessing an atmosphere similar to that of Jupiter, etc.
- 15) This study has illustrated the need for further development of tools that include PQ effects in the study of multiple-planet mission navigation.

7.2.3 Meaning of Results to Planetary Quarantine

The results of the large impactable analysis indicate that a multiple outer planet fly-by gravity assist mission, of the type analyzed, will have to include biasing in the navigation plan if it must be accepted that the probability of contamination given impact is one. Fuel penalties could prove to be significantly large. Therefore, it would seem that future areas of concentration should include the possibility of microbial burden reduction due to the effects of the natural environments.

7.3 PROBLEM AREA

The main problem area encountered so far is the lack of navigation and maneuver analysis data on multiple outer planet mission. Such data has been generated in this task for purposes of analysis.

7.4 FUTURE ACTIVITIES

7.4.1 Work Planned for Next Six Months

The following task will be completed in the next six months.

7.4.1.1 Preliminary Jupiter-Uranus-Neptune Mission PQ Analysis. Perform a PQ analysis to identify problem areas significantly different from a J-S-P mission. For instance, the greater distances involved lead to larger navigation uncertainties than those currently anticipated for a J-S-P mission. Also, there is a greater amount of uncertainty in the state of knowledge of the outer two giant planets than for Jupiter and Saturn.

The following tasks will be started in the next six months:

- 1) Study of entry heating of spacecraft or related debris into an outer planet atmosphere. The heating associated with atmospheric entry (Jupiter or Saturn) may be sufficient to render a spacecraft or related debris both internally and externally sterile by the time it reaches regions of the atmosphere that are of biological interest. Previous studies have not paid sufficient detail to various types of shapes, sizes, and materials of impacting bodies. Also, ablative characteristics were not considered in sufficient detail to understand effects of heat blockage.
- 2) Provide values of environmental parameters for Uranus, Neptune, and Pluto, and satellites of the outer planets. The Natural Space Environments Group at JPL is preparing a monograph of the planets Uranus, Neptune and Pluto. The results of this effort ought to be adapted for use in PQ analyses for outer planet missions. This is analogous to the use of monographs on the interplanetary environments, and Jupiter and Saturn for this purpose on the current task, and reported in a separate document.

They will also provide available parameters for the satellite of the outer planets.

- 3) Analyze problem areas not considered in the current J-S-P PQ analysis. The analyses would be based upon and complement current (fiscal 1970) J-S-P analyses, with the use of selected optimal mission strategies. It would also use up-dated spacecraft, launch vehicle, and mission parameters.
 - a) Include PQ analysis for satellites most likely to have biological interest. A great deal of interest is being shown in selecting trajectories for a J-S-P mission which will bring the spacecraft close to the satellites of Jupiter and Saturn.
 - b) Consider the problem of satisfying conflicting PQ constraints (if any exist) such as a planet and its satellites.
 - c) Complete particulate debris analysis, especially the determination of critical periods in the trajectory for transport of debris for specific ranges of physical parameters to the planet of interest. The probability of encountering Saturn's rings by a fly-by spacecraft should be determined and reflected in the particulate debris analyses. A particulate debris analysis should also be performed for the launch vehicle. The hazards of flying through the asteroid belt and Saturn's rings should be considered in an analysis of the probability of S/C disintegration.

7.4.2 Additional Required Work

The following work is required to better understand the problems of planetary quarantine analyses for outer planet missions and is an addition of the work described in para. 7.4.1. Included there were starts on tasks, to be completed later, which also fit into the category of tasks considered here.

7.4.2.1 Preliminary Jupiter Orbiter and/or Entry Probe Mission PQ Analysis.

A PQ analysis similar to that currently being performed for a J-S-P mission. The fact that an orbiter will spend a longer time in the vicinity of the planet coupled with different modes of entry into the planetary atmosphere from a

fly-by requires a separate analysis for such a mission. Even if an entry probe would be sterilized, an analysis would still be in order to minimize the sterilization cycle. Furthermore, an analysis must be performed for the bus carrying the probe.

7.5 PUBLICATIONS

Stavro, W. and Gonzalez, C., "Planetary Quarantine Considerations for Outer Planet Mission", Preprint No. AAS-71-122, paper presented at the AAS 17th Annual Meeting, Seattle, Washington, June 28-30, 1971.

Stavro, W. and Gonzalez, C., "Flight Path and Mission Strategies to Satisfy Outer Planet Quarantine Constraints", Preprint No. AAS-71-319, paper presented at the AAS/AIAA Astrodynamics Specialists Conference, Ft. Lauderdale, Florida, August 17-19, 1971.

SECTION VIII
POST LAUNCH RECONTAMINATION STUDIES

NASA No. 191-58-63-11-00-55

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8.1 INTRODUCTION

A great deal of effort is devoted to the maintenance of spacecraft (S/C) cleanliness and the monitoring/control of the associated microbial burden throughout the S/C fabrication and assembly activities. In the case of a lander probe, sterilization requirements may be imposed on the lander assemblies, which in addition may have to be encapsulated in a sterile condition prior to launch.

The primary purpose of these activities is to minimize the probability of biologically contaminating the various planets via Earth probes in order to investigate the existence of authentic native life or life precursors on such planets.

During the launch and the spaceflight portions of a given mission, however, the spacecraft is exposed to a variety of environments. Such environments may cause the redistribution of viable particulate burden on the spacecraft by affecting the dislodgement/transport of such a burden.

The fundamental interests relative to the problem of environmentally induced recontamination in the case of a lander/orbiter mission are:

- 1) Overall S/C recontamination from viable burden on the payload and shroud cavity internal surfaces as well as particulate redistribution on the orbiter/lander external surfaces.
- 2) Recontamination of an initially sterile lander assembly due to migration of viable burden from a nonsterile orbiter after the lander is decapsulated in readiness for entry to a planet surface.

This study represents an effort to assess the effect of typical mission environments on possible viable particulate migration. Assessing the various

migration mechanisms and quantifying such effects represent the core of this effort. The goal is to develop a methodology and a quantitative analytical tool for the evaluation of the recontamination problem for various planetary missions and mission strategies.

8.2 SIGNIFICANT ACCOMPLISHMENTS

8.2.1 Launch Phase Recontamination

A probabilistic model was constructed for the launch phase recontamination. The model relates to the deposition on a spacecraft of microorganisms released from the shroud during the launch phase due to the launch dynamic environments. According to this model, the shroud is divided into N_z zones. The expected number of particles of diameter D_j from zone i that will migrate to the spacecraft as a result of dynamic event k is

$$E(i, j, k) = N_r(i, j, k) P_t(i, j, k) P_a(j) \quad (1)$$

where

$N_r(i, j, k)$ is the number of particles of diameter D_j released from region i during the k^{th} event

$P_t(i, j, k)$ is the probability that a released particle will traverse the space between the shroud and spacecraft and strike the spacecraft

$P_a(j)$ is the probability that a particle of diameter D_j will adhere to the spacecraft

The total expected number of particles adhering to the spacecraft is

$$E = \sum_{i=1}^{N_z} \sum_{j=1}^{N_r} \sum_{k=1}^N E(i, j, k) \quad (2)$$

If the average number of microorganisms per particle of diameter D_j is $N_p(j)$ the expected spacecraft microorganism burden is

$$B = \sum_{i=1}^{N_z} \sum_{j=1}^{N_r} \sum_{k=1}^N N_p(j) E(i, j, k) \quad (3)$$

8.2.1.1 Release Probability. The particle force model (Ref. 1) provides the probability of release of a particle as a function of the particle diameter. The number of particles of diameter d released from the shroud by the first dynamic event is

$$n(d) = n_o(d) \left[1 - \exp(-g/g_o)^t \right] \quad (4)$$

where g is the maximum acceleration of the surface and $n_o(d)$ is the number of particles of diameter d . The term t is given by

$$t = 0.731 - 0.00454 d \quad (5)$$

where d is the particle diameter in microns. The term g_o is given by

$$g_o = \frac{6S(C_1 + C_2 RH)}{\pi d^2 g} \quad (6)$$

where

$$S = 0.0331$$

$$C_1 = 0.4$$

$$C_2 = 0.006$$

RH = % relative humidity

$$\rho = \text{Particle density} - \text{g}\mu\text{m}^{-3}$$

$$g = \text{Acceleration of gravity at sea level} - 980 \text{ cm sec}^{-2}$$

For subsequent events, no particles are released if the acceleration is less than that experienced during any of the previous events. If the acceleration level is higher than all previous events, the number released is the difference between the number given by Eq. (4) and the number released in all previous events.

8.2.1.2 Transfer Probability. No satisfactory model that would yield a closed form solution was obtainable; however, order of magnitude analysis has shown that the probability of transfer is very low.

Particles released by the shroud with a finite velocity will be acted upon by drag forces given by

$$D = -(1/2)\rho_{\alpha} C_D A v^2 - 6\pi\eta a v \quad (7)$$

where

D = Drag force

ρ_{α} = Air density within the shroud

C_D = Pressure drag coefficient

A = Cross-sectional area of the particle

v = Particle velocity

η = Viscosity coefficient of air

a = Particle radius

For particles in the diameter range 10 - 100 μ m, it can be shown that, for reasonable ejection velocities (below 10⁶ cm sec⁻¹), the first term in Eq. (7) is negligible. Solving the differential equation

$$m \frac{dv}{dt} = -6\pi\eta a v \quad (8)$$

one obtains

$$v = v_o \left(1 - \frac{x}{x_o} \right) \quad (9)$$

where

$$x_o = \frac{mv_o}{6\pi\eta a} \quad (10)$$

Note that in Eq. (9) the particle comes to rest in a distance x_o . Since

$$m = (1/6) \pi \rho d^3 \quad (11)$$

$$a = (1/2) d \quad (12)$$

where

ρ = Particle density

d = Particle diameter

Using

$\rho = 1 \text{ g cm}^{-3}$ (for the particle)

$\eta = 1786 \times 10^{-7} \text{ g cm}^{-1} \text{ sec}^{-1}$ (for air)

Eq. (10) becomes

$$x_o = 3.1 \times 10^2 v_o d^2 \quad (13)$$

This distance is plotted in Fig. 8-1 as a function of release velocity for diameters of 10 μm and 100 μm , respectively. For intermediate diameters, the curve would fall between these two.

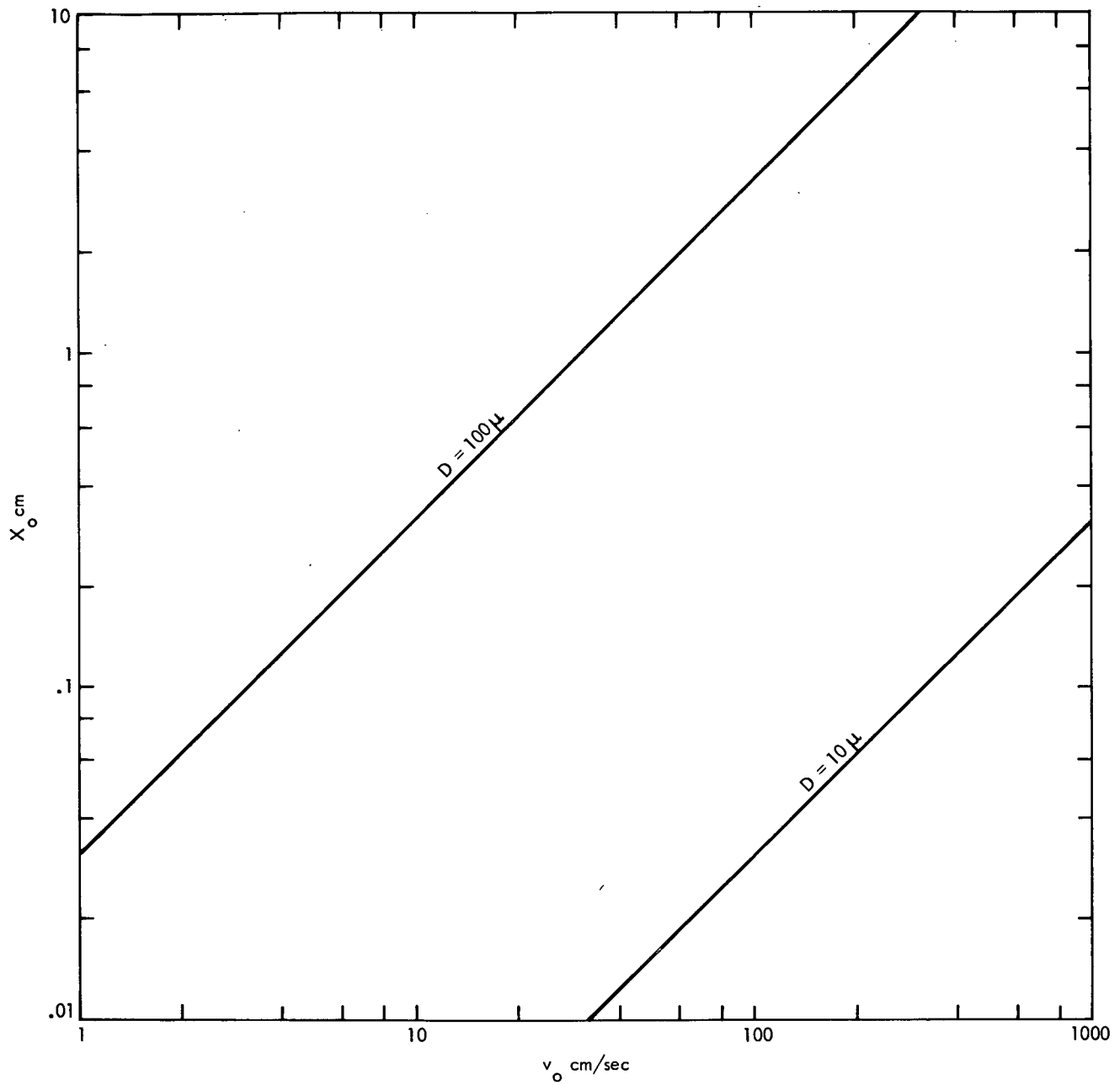


Fig. 8-1. Decay distance vs initial velocity of particles ejected within the shroud in stagnant air

Thus, it is apparent that few or no particles will go directly from the shroud to the spacecraft in a stagnant atmosphere. They will be trapped in the shroud cavity. Air currents within the cavity will predominantly sweep these particles out the exhaust ports, deposit them on the spacecraft or shroud surface, or circulate them until the time of shroud release, depending substantially on the method of venting the shroud and the flow pattern that materializes due to the location of the vent holes.

8.2.1.3 The Program. A computer program was written to compute the recontamination probability based on the initial microbial/particulate burden on the shroud following the proposed models. This program is written in FORTRAN IV language and, though checked out on the UNIVAC 1108 computer, it should work with little or no modification on any machine with a FORTRAN IV compiler. A description and listing of the subject program is given in Appendix A.

8.2.2 Spaceflight Phase Particulate Redistribution Mechanisms

Dynamic events of relevance were identified as those due to meteoroid impact, pyro-firings, and the various deployment maneuvers. Mid-course engine burns, however, do not seem to create sufficient acceleration levels to release a significant number of ejecta. Typical acceleration levels due to engine burn on the previously flown spacecraft, namely the Mariner Mars '69 and the Mariner Mars '71, are below .05 g's RMS.

8.2.2.1 Micrometeoroid Impact. In conjunction with Dr. Charles Babcock of Caltech, a summary of hypervelocity impact models was prepared and evaluated with an assessment made regarding the applicability of various models to analytical solution. The models representative of spacecraft structures selected for analytical treatment are:

- 1) Infinite plate with pressure/surface loading
- 2) Infinite plate with perforation/shear loading
- 3) Semi-infinite solid with pressure/surface loading
- 4) 2-layer laminated plates in items 1) and 2) above.

A reference list has been prepared containing analytical techniques to be used as the basis for the solution of these models. The reference list is presented as References 3 thru 13.

In addition to the analytical approach defined above, initial hypervelocity test plans and concepts were formulated in conjunction with Langley Research Center (LRC) personnel.

This test series is designed to provide the necessary data for the verification of the hypervelocity impact analytical models, and additional data for the particle ejecta force model. The overall test data will also enable particle release estimates to be made directly from meteoroid physical parameters for particular materials, boundary conditions, and projectile properties (mass, diameter, and velocity).

8.2.2.2 Velocity of Ejection Due to a Micrometeoroid Impact and Pyro Shock Environments. A knowledge of the velocity of ejected particles from spacecraft surfaces during space flight is essential for describing particle trajectories around the spacecraft for recontamination analysis. That is, if the velocity of the ejecta is high enough, particles will follow straight line paths and may escape from the vicinity of the spacecraft. On the other hand, if the velocity of the ejecta is small enough, the spacecraft electrostatic force, gravitational force, and other forces, may be great enough to retain the ejecta in a cloud around the spacecraft that may eventually redeposit on the various surfaces.

As indicated earlier, dynamic events in space of large enough amplitude to dislodge a significant number of particles include pyro events and meteoroid impact. In the case of meteoroid impact, a qualitative representation of the acceleration/velocity time history of a point on the surface in the vicinity of impact is shown in Fig. 8-2.

The acceleration shown is a damped wave with decreasing frequency. The initial acceleration is positive; that is, the surface accelerates toward the particle. Not until the acceleration assumes a negative value (point a) can a particle be released. Furthermore, the adhesion force model (Ref. 1) predicts all particle removal will occur between point a and the maximum negative acceleration at point b. At point a the velocity is a maximum. Somewhere between points b and d, say at point c, the velocity reaches zero.

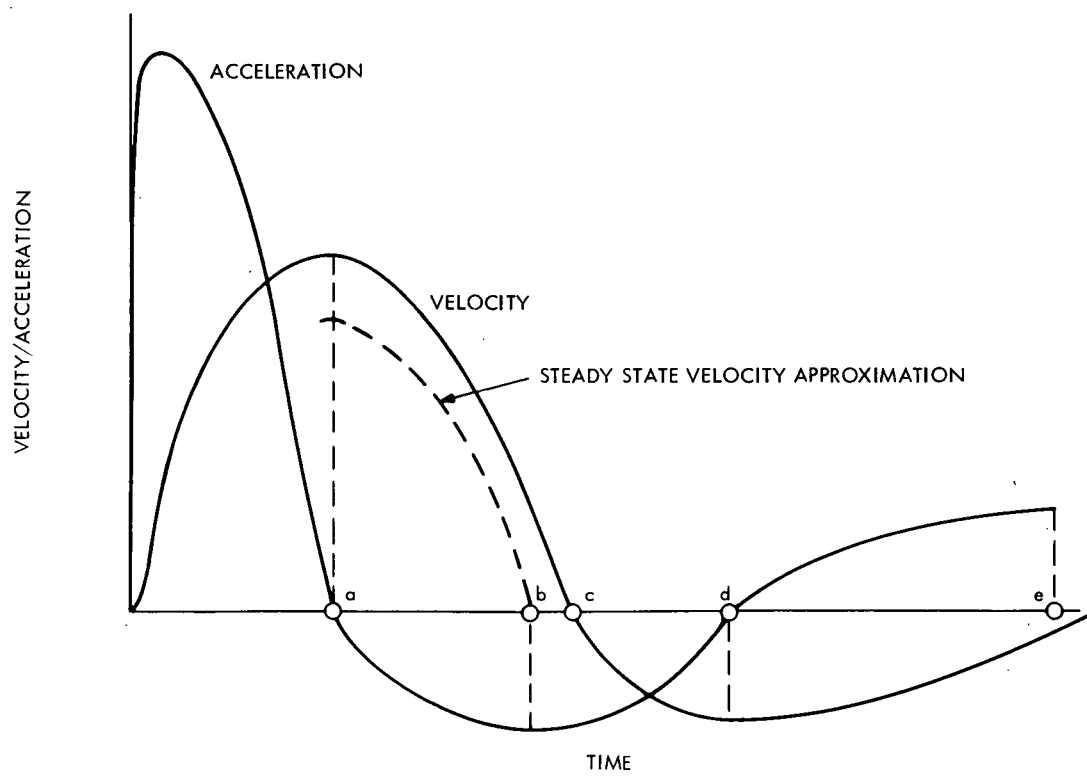


Fig. 8-2. Shock wave velocity/acceleration time history of a surface point

It is assumed that between points a and b the motion can be approximated by a steady state excursion with acceleration amplitude equal to that at time b. This assumption is conservative in that the velocity will reach zero at point b. This implies that, for a particle released at any given acceleration level, the velocity of ejection (v_{ejc}) will be somewhat lower than in the actual case.* The equations relating displacement, velocity, and acceleration for simple harmonic motion are given in Eqs. (14), (15), and (16).

$$x = A \sin \omega t = x_{\max} \sin \omega t \quad (14)$$

$$v = A \omega \cos \omega t = v_{\max} \cos \omega t \quad (15)$$

$$a = -\omega^2 A \sin \omega t = -a_{\max} \sin \omega t \quad (16)$$

Pyro events lend themselves to an equivalent analysis. However, the initial acceleration may be negative. If it is, particles cannot be released with a velocity away from the surface until point d. Point d is equivalent to point a in the case of positive initial acceleration and particles are released within the period from point d to e.

8.2.2.3 Computations. Typical spacecraft dynamic environment estimates are given for various types of events in Table 8-1. In each case, peak accelerations (g_{peak}) corresponding to the first maximum of Fig. 8-2 are given. For the first three entries, typical frequencies are given enabling one using Eqs. (14), (15), and (16) to solve for peak displacement and velocities. The final entry concerning meteoroid impact is an order of magnitude estimate.

The damping factor from cycle to cycle for pyro events is about 0.5 to 0.9. At this point, the g_{\max} 's obtained enabled one to calculate the maximum percentage of the total initial particle burden removed from a surface (P_{\max}).**

*The smaller v_{ejc} is, the greater chance the small forces associated with the spacecraft have of recapturing the particles and recontaminating the spacecraft.
 **For a given particle diameter.

Table 8-1. Estimated peak acceleration, displacement, and nominal frequencies

Dynamic Environment	f(Hz) Frequency	g_{peak} (g's) Acceleration	A(cm) Displacement	v_{max} (cm sec ⁻¹) Velocity
Pyro events at Joint	2000-5000	2000	0.0124-0.00199	156.0 62.5
Pyro events	2000	500	3.10×10^{-3}	39.0
Separation Launch Vehicle bio canister	5000	3000	0.00298	93.6
Meteoroid ^a	60000	10^5 ^a	0.00069	260
^a Order of magnitude estimates				

$$P_{\text{max}} = 100 \left\{ 1 - \exp \left[- \left(\frac{g_{\text{max}}}{g_0} \right)^t \right] \right\} \quad (17)$$

For meteoroid impact the damping factor is not yet known. Since the time relationship between the acceleration and velocity is assumed to be that of the $\sin \omega t$ and $\cos \omega t$, one may write acceleration as a function of velocity and g_{max} .

$$v/v_{\text{max}} = \cos \omega t \quad (18)$$

$$\sin \omega t = \left\{ 1 - (v/v_{\text{max}})^2 \right\}^{1/2} \quad (19)$$

$$g = g_{\text{max}} \left\{ 1 - (v/v_{\text{max}})^2 \right\}^{1/2} \quad (20)$$

Therefore, the percentage of the total number of particles removed on the surface can be written as in Eq. (21)*

$$P = 100 \left[1 - \exp \left(- \left[\frac{g_{\max}}{g_o} \left\{ 1 - (v/v_{\max})^2 \right\}^{1/2} \right]^t \right) \right] \quad (21)$$

which indicates that given a velocity v the percentage P of the total original number of particles on the surface will be ejected with a velocity v_{ejc} satisfying inequality Eq. (22).

$$v \leq v_{\text{ejc}} \leq v_{\max} \quad (22)$$

Of more interest, however, is the percentage of the total number of particles that will be ejected at g_{\max} that have velocities satisfying inequality Eq. (23).

$$0 \leq v_{\text{ejc}} \leq v \quad (23)$$

These values are tabulated in Table 8-2.

8.3 PROBLEM AREAS

8.3.1 Surface Response to Micrometeoroid Impact

The problem of quantitatively defining the surface response surrounding a meteoroid impact has not been completed. The unforeseen lack of directly applicable reference material and the complexity of the subject have delayed the solution of this problem.

The modifications necessary to existing analytical treatment of this subject and hypervelocity impact tests to be conducted for analytical verification are described in para. 8.4.

*For a given particle diameter.

Table 8-2. P_L or percentage of particles released satisfying $0 \leq v_{ejc} \leq v$

$D_p = 100\mu m$							
	g_{max}	10	100	1000	10^4	10^5	10^6
v/v_{max}	P_{max}^a	23%	40%	62%	84%	97%	100%
0.9		18%	16%	13.5%	8.8%	3.4%	0.4%
0.5		3.4%	3.0%	2.4%	1.4%	0.5%	0.04%
0.3		1.1%	1.0%	0.78%			
0.1		0.12%	0.11%	0.083%	0.05%	0.016%	0.001%
0.01		0.0012%	0.0011%	0.0008%	0.0005%	0.0002%	$1.4 \times 10^{-5}\%$
$D_p = 10\mu m$							
	g_{max}	10	100	1000	10^4	10^5	10^6
v/v_{max}	P_{max}^a	0.16%	0.79%	3.7%	17%	59%	99%
0.9		43%	43%	42%	41%	33%	7.3%
0.5		9.4%	9.4%	9.2%	8.6%	6%	0.66%
0.3		3.2%	3.2%	3.1%	2.9%	2.0%	0.19%
0.1		0.34%	0.34%	0.31%	0.31%	0.21%	0.02%
0.01		0.0034%	0.0034%	0.0034%	0.0031%	0.002%	$1.94 \times 10^{-4}\%$
P_{max}^a is the maximum percentage released of the total initial number of particles on the surface for the given g_{max} .							

8.3.2 Particle Forces in Space

To determine the trajectories of ejected particles in space, one needs detailed information of the forces acting on the free particles. An order of magnitude analysis indicated that the electrostatic force may be the most significant force in space.

The first step in analyzing this force was to determine the surface potentials on the spacecraft. The approach to this problem assumed the spacecraft to be a spherical conductor. The solar wind plasma was modeled as a stream of electrons and ions traveling radially outward from the Sun. Equations were derived for the electron and ion current to the spacecraft by finding the capture radius of the particles in the electric field of the spherical spacecraft model. An attempt was made to calculate photoelectron current based on the photon flux as a function of the photon energy, the quantum efficiency of the photons, and the potential of the sphere. This approach has 3 primary weaknesses: 1) there is inadequate information concerning quantum efficiencies of spacecraft surfaces; 2) the spacecraft is not a conducting sphere; and 3) plasma currents are more complicated than those used.

8.4 FUTURE ACTIVITIES

8.4.1 Micrometeoroid Impact

8.4.1.1 Analysis. The analytical solution of four basic hypervelocity impact problems (as described in para. 8.2.2.1) is being pursued by JPL Division 35 under the cognizance of Mr. Al Knoell. The result of this study will be the definition of surface response utilizing a computer model of meteoroid impact.

8.4.1.2 Testing for Verification. The analytical solution to the surface response to meteoroid impact will be verified utilizing data obtained from the test series of hypervelocity impacts at LRC.

The force model of particulate release will be verified, or modified if necessary, utilizing a series of vacuum chamber shock tests at JPL. This test series will be conducted to determine the effect of shock (surface

acceleration) intensity, relative humidity, and other pertinent environments on particle release parameters (the force model described in Ref. 1).

8.4.1.3 Particle Forces in Space. From a review of the literature, it became apparent that there is not enough data to solve the photoelectron current problem theoretically and that existing experimental values will have to be used. Forms of equations have been chosen for the plasma electron and ion currents. The three currents - photoelectron, plasma ion, and electron - combine to form an equilibrium problem which may be solved for a surface potential at which the sum of the currents to the surface point is zero.

It will also be necessary to formulate the boundary value electrostatic Dirichlet problem. That is, the potentials at each point on the surface of the spacecraft are specified, and we determine the potential everywhere in the vicinity of the spacecraft. Because of the complex geometry of an actual spacecraft, the problem cannot be solved in closed form and must be approached numerically. It has been decided that the appropriate approach will be to divide the spacecraft into regions of different surface charge density.

In each of these regions, a point is given at which the potential is specified on the surface. The number of points and regions determines the accuracy obtained. Coulomb's law and the given potentials form a set of equations through which one may determine the surface charge densities that satisfy the given boundary conditions. Once the surface charge densities have been determined, one may calculate the electrostatic potential anywhere in the vicinity of the spacecraft.

The potential of the particles in space is very important because this determines the magnitude and direction of a force on the particles. There are two regions of interest in which the particle will experience different environmental conditions: 1) the sunlit region where all three currents, photoelectron, ion plasma, and electron, will be collected by the particle; and 2) the dark region. Preliminary analysis would indicate that the particle will attain a positive potential in the sunlit region. In the dark region, the only current incident on the particles is the plasma photoelectron, and one would expect the particles to become negative.

A major question of interest is the charging rates of these particles. That is, if the particle travels from a sunlit region to a dark region, how long will it take the particle to become negative? Or vice versa; how long would it take a particle in the dark region entering a sunlit region to become positive? This question is of importance for the following case in point.

Assume the lander portion of a spacecraft is negative because it is shaded. An ejected particle in the Sun is positive. If it travels to the vicinity of the dark region, it will be attracted. If, however, it enters the shade and becomes negative, it will experience a repulsive force and may not be a recontamination problem. Therefore, the charging rates of the particles in space in the dark and sunlit regions are important when compared to the transit times of these particles. These topics will be pursued in the next 6 months' effort.

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APPENDIX A

LAUNCH PHASE RECONTAMINATION MODEL COMPUTER
PROGRAM DESCRIPTION AND LISTING

A.1 INPUT

The number of events and number of zones in the model, up to a maximum of ten each, are read from a punched card (Format 2I10).

The average number of microbes per particle as a function of particle diameter are read next. The first data point is for particles in the range 10-11 micrometers, etc., for a total of 90 data points (Format 10E8.2).

The initial particle burden as a function of particle size for each zone to read next (Format 10E8.2). There are 90 data points for each zone.

The maximum acceleration level in each zone for the first event is read (Format 10E8.2). The next card is the acceleration in each zone for the second event, etc.

Finally, the probabilities of transfer for each event and zone (Format E8.2). The first card reads that of the first zone, first event. The second card reads first zone, second event, etc.

A.2 COMPUTATIONS

For each event and zone the program computes:

- 1) The transfer probability P_t . This is done in a separate subroutine. At present, this is a dummy routine which simply reads the value from cards.
- 2) The adherence probability P_a . This is also done in a dummy subroutine which sets the probability equal to unity.* The purpose of these routines is to provide ease of update as the model becomes more sophisticated.
- 3) The fraction of particles released by the last previous event.

*A conservative number from a PQ standpoint.

- 4) The fractions of particles released by the present event if the present acceleration value exceeds all previous values in the zone.
- 5) The expected number of particles in each diameter range (Eq. (1), para. 8.2.1) contaminating the spacecraft.
- 6) The total number of microbes reaching the spacecraft.

The program then calculates the sum over zones of microbial burden for each event and the sum over events for each zone and the total burden.

Finally, the total number of particles of each size transferred to the spacecraft is computed.

A.3 OUTPUT

The output from this program is two tables.

Table A-1 gives the number of organisms reaching the spacecraft as a result of each event in each zone. It also gives the expected total number of transferred organisms.

Table A-2 gives the expected total number of particles in each diameter range migrating to the spacecraft. The purpose of including Tables A-1 and A-2 is to delineate the format and not to convey any quantitative conclusions on any mission.

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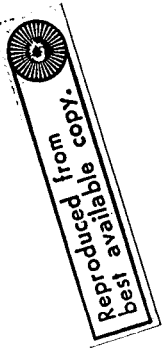
Fig. A-1. Program list

```

C      TE - SUM OVER ALL EVENTS OF CONTAMINATION FOR EACH ZONE      MAIN0280
C      TEZ - TOTAL CONTAMINATION FOR EACH EVENT AND ZONE           MAIN0290
C      TZ - SUM OVER ALL ZONES OF CONTAMINATION FOR EACH EVENT     MAIN0300
C      TN - NO ORGANISMS FROM EACH EVENT, ZONE CONTAMINATING THE SAC MAIN0310
C                                                                    MAIN0320
C                                                                    MAIN0330
C      DIMENSION DENMIC(90),IN(10,50,10),DPN(10,90),GM(10,10),F(90), MAIN0340
      IFL(90),TEZ(10,10),NT(10),TE(10),TZ(10),VI(9),VZ(7),FR(30) MAIN0350
      DATA VI/('','','X','H','Z','N','E','/','/','/'), MAIN0360
      DATA VZ/('10X','5','SEVENT','5X',' ','112','3X',' ','5H','10','1AL','/','/'), MAIN0370
      DATA N/1,2,3,4,5,6,7,8,9,10/ MAIN0380
C                                                                    MAIN0390
C      READ NC.EVENTS, ZONES                                         MAIN0400
      READ (5,1000) NE,NZ                                         MAIN0410
C                                                                    MAIN0420
C      READ MICROBE DENSITY                                          MAIN0430
      READ (5,1001) DENMIC(J),J=1,90) MAIN0440
C                                                                    MAIN0450
C      READ PARTICLE DENSITY FOR EACH ZONE                          MAIN0460
      DO 10 I=1,NZ                                                 MAIN0470
10 READ (5,1001) (DPN(I,J),J=1,70) MAIN0480
C                                                                    MAIN0490
C      READ MAX ACCEL LEVEL FOR EACH ZONE, EVENT                   MAIN0500
      DO 20 K=1,NE                                                 MAIN0510
20 READ (5,1001) (GM(I,K),I=1,90) MAIN0520
C                                                                    MAIN0530
C      COMPUTE EXPECTED NO PARTICLES PER EACH ZONE AND EACH EVENT MAIN0540
C                                                                    MAIN0550

```

Fig. A-1. Program list (contd)



```

C      INITIALIZE FRACTION RELEASED
      DO 60 I=1,NZ
      DO 30 J=1,30
      30 F(J)=0.0
      G=0.0
      DO 50 K=1,NE
C
C      COMPUTE TRANSFER PROBABILITY
      CALL TRPRCB(I,K,PT)
C
C      COMPUTE ADHERENCE PROBABILITY
      CALL ADPRCB(I,K,PA)
C
C      IF ACCEL LEVEL IS NOT HIGHER THAN PREVIOUS NO PARTICLES ARE RELEASED
      IF (GM(I,K).L.E.G) GO TO 50
      40 FL(J)=F(J)
C
C      COMPUTE FRACTION RELEASED IN LAST PREVIOUS EVENT
      DO 40 J=1,30
      40 FL(J)=F(J)
C
C      COMPUTE RELEASE PROBABILITY
      CALL REPRCB(GMT,K),FI
C
C      COMPUTE NO PARTICLES RELEASED FROM THE KTH ZONE DURING THE ITH
      EVENT AS FCN OF PARTICLE CHARACTER
      DO 50 J=1,30
      50 IN(I,J,K)=(F(J)-FL(J))*PI*PA*GM(I,K)
C
      MAIN0560
      MAIN0570
      MAIN0580
      MAIN0590
      MAIN0600
      MAIN0610
      MAIN0620
      MAIN0630
      MAIN0640
      MAIN0650
      MAIN0660
      MAIN0670
      MAIN0680
      MAIN0690
      MAIN0700
      MAIN0710
      MAIN0720
      MAIN0730
      MAIN0740
      MAIN0750
      MAIN0760
      MAIN0770
      MAIN0780
      MAIN0790
      MAIN0800
      MAIN0810
      MAIN0820
      MAIN0830

```

Fig. A-1. Program list (contd)

```

C
C      COMPUTE MAXIMUM ACCEL
      ED G=AMAX1(G,GX(I,K))
C
C      COMPUTE TOTAL NO MICROEES RELEASED FROM THE WITH ZONE IN THE ITH
      EVENT
      DO 70 I=1,NZ
      DO 70 K=1,NZ
      DO 70 J=1,30
      70 IZ(I,K)=IZ(I,K)+IN(I,J,K)*DENW(I,CJ)
C
C      LOCATE HEADINGS FOR TABLE DEPENDING ON NUMBER OF COLUMNS
      GO TO (71,72,73,74,75,76,77,78,79,81),NZ
      71 VI(2)=425*
      V2(4)=41*
      GO TO 82
      72 VI(2)=471*
      V2(4)=42*
      GO TO 82
      73 VI(2)=436*
      V2(4)=42*
      GO TO 82
      74 VI(2)=442*
      V2(4)=44*
      GO TO 82
      75 VI(2)=447*
      V2(4)=45*
      GO TO 82

```

Fig. A-1. Program list (contd)

76 V1(2)=53.
 V2(4)=0.
 GO TO 82
 77 V1(2)=58.
 V2(4)=7.
 GO TO 82
 78 V1(2)=54.
 V2(4)=18.
 GO TO 92
 79 V1(2)=69.
 V2(4)=9.
 GO TO 82
 81 V1(2)=75.
 V2(4)=10.
 C
 C WRITE TABLE HEADINGS FOR CONTAMINATION BY EVENT AND ZONE
 82 WRITE (5,1002)
 WRITE (6,1003)
 WRITE (6,1004)
 WRITE (6,1005)
 WRITE (6,1006)
 WRITE (6,1007)
 WRITE (6,1008)
 WRITE (5,1009) (N(I),I=1,N2)
 C
 C COMPUTE CONTAMINATION FOR EACH EVENT, ALL ZONES AND EACH ZONE.
 C ALL EVENTS
 DO 30 I=1,N2
 DO 30 K=1,NZ

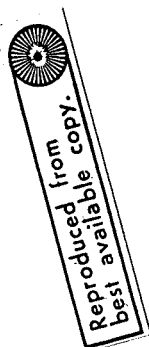


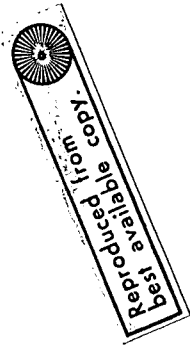
Fig. A-1. Program list (contd)

```

      TZ(I)=TZ(I)+TEZ(I,K)
      90 TE(K)=TE(K)+TEZ(I,K)
      C
      C   WRITE TABULATED DATA
      DO 90 K=1,NE
      90 WRITE (6,1008) K,(TEZ(I,K),I=1,NZ),TE(K)
      WRITE (6,1009) (TZ(I),I=1,NZ)
      C
      C   COMPUTE AND WRITE TOTAL CONTAMINATION ALL EVENTS, ZONES
      DO 100 I=1,NZ
      100 T=1+TZ(I)
      WRITE (6,1010) T
      C
      C   COMPUTE FINAL SPACECRAFT BURDEN AS Fcn OF PARTICLE DIAMETER
      DO 110 I=1,NZ
      DO 110 J=1,30
      DO 110 K=1,NE
      110 FR(I)=R(J)*TN(I,J,K)
      C
      C   WRITE FINAL BURDEN TABLE
      WRITE (6,1002)
      WRITE (6,1003)
      WRITE (6,1011)
      WRITE (6,1012)
      WRITE (6,1013)
      WRITE (6,1014)
      DO 120 L=1,15
      120 L=L+1

```

Fig. A-1. Program list (contd)



```

L2=L+1
L3=L+24
L4=L3+1
L5=L+39
L6=L5+1
L7=L+54
L8=L7+1
L9=L+63
L10=L9+1
L11=L+84
L12=L11+1

120 WRITE(6,1015) L1,L2,FR(L),L3,L4,FR(L+15),L5,L6,FR(L+30),L7,L8,
1 FR(L+45),L9,L10,FR(L+60),L11,L12,FR(L+75)

C
1000 FORMAT(2I10)
1001 FORMAT(10E6.2)
1002 FORMAT(11,50X,'SPACECRAFT RECONTAMINATION MODEL',/)
1003 FORMAT(61X,'LAUNCH PHASE',////)
1004 FORMAT(49X,'EXPECTED NUMBER OF ORGANISMS RELEASED')
1005 FORMAT(43X,'FROM EACH ZONE AND CONTAMINATING THE SPACECRAFT')
1006 FORMAT(55X,'FOR ALL DYNAMIC EVENTS',////)
1008 FORMAT(7,10X,15,10(3X,1PEP.2))
1009 FORMAT(7,10X,'TOTAL',10(3X,1PEP.2))
1010 FORMAT(7,10X,'THE EXPECTED OVERALL TRANSFER OF BURDEN IS',1PEP.2,MAIN1910
1, ' MICRO-ORGANISMS')
1011 FORMAT(6X,'FINAL SPACECRAFT PARTICULATE BURDEN')
1012 FORMAT(49X,'FOR PARTICLES 10-100 MICRONS IN DIAMETER',/)
1013 FORMAT(61X,'PARTICLE',3X,'NUMBER',2X)

MAIN1680
MAIN1690
MAIN1700
MAIN1710
MAIN1720
MAIN1730
MAIN1740
MAIN1750
MAIN1760
MAIN1770
MAIN1780
MAIN1790
MAIN1800
MAIN1810
MAIN1820
MAIN1830
MAIN1840
MAIN1850
MAIN1860
MAIN1870
MAIN1880
MAIN1890
MAIN1900
MAIN1910
MAIN1920
MAIN1930
MAIN1940
MAIN1950

```

Fig. A-1. Program list (contd)

Fig. A-1. Program list (contd)

SUBROUTINE REPROBIS1,F
 C
 C
 C THIS SUBROUTINE COMPUTES THE PROBABILITY OF RELEASE OF PARTICLES
 C AS A FCN OF PARTICLE DIAMETER
 C BASED ON THE MODEL OF C. HALGENSCHILD
 C SEE ICM 2345 - 2152
 C
 C
 C
 DIMENSION F(30)
 S=.03
 CL=.04
 C2=.006
 RH=50.
 R=2.5E-12
 G=980.
 DO 10 I=1,30
 DP=I+.9
 T=.731-.00454*DP
 CC=(6.*S*(CL+C2*PH))/((3.14159*DP**2*P*C)
 10 F(I)=1.-EXP(-(GI/501**T)
 RETURN
 END

15X2

2
3

[illegible]

Fig. A-1. Program list (contd)

[illegible]

Fig. A-1. Program list (contd)

1.00E+001.00E-061.00E+00
 1.00E+001.00E-061.00E+00
 1.00E+001.00E-061.00E+00
 1.00E+001.00E-061.00E+00
 1.00E+001.00E-061.00E+00

23

Fig. A-1. Program list (contd)

Table A-1. Expected number of organisms released from each zone and contaminating the spacecraft for all dynamic events

Event	Zone		Total
	1	2	
1	1.52-06	1.76-05	1.91-05
2	1.61-05	0.00	1.61-05
3	0.00	2.01-05	2.01-05
Total	1.76-05	3.77-05	
The expected overall transfer of burden is 5.53-05 microorganisms.			

Table A-2. Output example indicating expected total number of particles in diameter range migrating to the spacecraft

Particle diameter	Number particles	Particle diameter	Number particles	Particle diameter	Number particles	Particle diameter	Number particles	Particle diameter	Number particles	Particle diameter	Number particles	Particle diameter	Number particles	Particle diameter	Number particles	Particle diameter	Number particles
10- 11	5.00-08	25- 26	2.37-07	40- 41	4.50-07	55- 56	6.54-07	70- 71	8.30-07	85- 86	5.74-07						
11- 12	6.82-03	26- 27	2.50-07	41- 42	4.64-07	56- 57	6.66-07	71- 72	8.41-07	86- 87	9.82-07						
12- 13	7.80-08	27- 28	2.64-07	42- 43	4.78-07	57- 58	6.79-07	72- 73	8.51-07	87- 88	9.91-07						
13- 14	8.83-08	28- 29	2.78-07	43- 44	4.92-07	58- 59	6.91-07	73- 74	8.61-07	88- 89	9.99-07						
14- 15	9.90-08	29- 30	2.92-07	44- 45	5.06-07	59- 60	7.04-07	74- 75	8.72-07	89- 90	1.01-06						
15- 16	1.10-07	30- 31	3.07-07	45- 46	5.20-07	60- 61	7.16-07	75- 76	8.02-07	90- 91	1.01-06						
16- 17	1.22-07	31- 32	3.21-07	46- 47	5.34-07	61- 62	7.26-07	76- 77	8.92-07	91- 92	1.02-06						
17- 18	1.33-07	32- 33	3.35-07	47- 48	5.48-07	62- 63	7.40-07	77- 78	9.01-07	92- 93	1.03-06						
18- 19	1.45-07	33- 34	3.49-07	48- 49	5.61-07	63- 64	7.52-07	78- 79	9.11-07	93- 94	1.04-06						
19- 20	1.58-07	34- 35	3.64-07	49- 50	5.75-07	64- 65	7.63-07	79- 80	9.20-07	94- 95	1.04-06						
20- 21	1.70-07	35- 36	3.78-07	50- 51	5.38-07	65- 66	7.75-07	80- 81	9.30-07	95- 96	1.05-06						
21- 22	1.83-07	36- 37	3.92-07	51- 52	6.01-07	66- 67	7.86-07	81- 82	9.39-07	96- 97	1.06-06						
22- 23	1.96-07	37- 38	4.07-07	52- 53	6.15-07	67- 68	7.97-07	82- 83	9.48-07	97- 98	1.07-06						
23- 24	2.10-07	38- 39	4.21-07	53- 54	6.28-07	68- 69	8.08-07	83- 84	9.57-07	98- 99	1.07-06						
24- 25	2.23-07	39- 40	4.35-07	54- 55	6.41-07	69- 70	8.19-07	84- 85	9.65-07	99- 100	1.08-06						